At the roots of limbed vertebrate diversity: new light on patterns and processes of early tetrapod evolution

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Abstract

The origin of limbed vertebrates (tetrapods) from their fish-like ancestors is an iconic example of a major evolutionary radiation. The group of vertebrates that covered this transition, the early tetrapods, encompassed 12-15 groups, ranging from the Late Devonian to the Middle Cretaceous. The tempo and mode of the evolutionary diversification undergone by these organisms, however, remains largely unknown. To fill this gap in our understanding of the early proliferation of vertebrates, the core objective of the project is to quantify models of skeletal transformation across the transition from fish-like ancestors to early tetrapods in light of a revised, amended, and expanded database of cranial traits. Studies so far have relied on a limited database of osteological characters, often with few characters for certain complexes or a reduced taxon set for tetrapods over the transition. Here, I create a new database that will be used to retrieve a tree for the group which will be subjected to macroevolutionary analyses, answering questions concerned with rate and disparity. The retrieved tree shows a well resolved pattern of taxa with groupings that have been found previously. New taxa have impacted the tree tremendously pushing the divergence between the stem-amniotes and stem-amphibians backwards. The analysis of disparity reveals that phylogenetic diversification resulted in higher degrees of morphological disparity among clades relative to the degrees of within clade variation. Both the rate and disparity analyses indicate that early tetrapods show a burst of evolution at the start of the lineage and also at the amphibian-amniote split. This study has shed light upon how the early tetrapod group has evolved, revealing that these organisms underwent a degree of rapid diversification at the beginning of the clade across both analyses as well as creating a new tree for the group. The next step would be to add post cranial characters to the dataset allowing an even more comprehensive look at the early tetrapod radiation. Overall, my research contributes to appreciate the patterns and rates underlying the early proliferation of tetrapods, which is a fundamental step to advance our understanding of the subsequent diversification of modern tetrapods and their morphological complexities.

1. Introduction

Limbed vertebrates, otherwise known as tetrapods, are an extremely diverse and complex animal group, and include today's amphibians, reptiles, birds, and mammals. They do however all share an important feature, the possession of four limbs (unless secondarily lost), with the term tetrapod simply meaning "four feet" (Schoch, 2014). Understanding the relationships between early tetrapods is vital to understand our own evolution and position in the Tree of Life, as well as giving a unique perspective on the origin of a complex body plan and a major radiation (Ruta *et al.,* 2006). This origination of tetrapods from their fish-like ancestors encapsulates themes relevant to all biologists, including investigating patterns of diversification and the impact of key evolutionary events upon speciation. Also, understanding the early diversification and evolution of tetrapods is key to understanding how modern patterns of global biodiversity were shaped via the contribution of these organisms to the assembly of ecosystems.

The tetrapod total-group clade includes finned and limbed taxa that are more closely related to extant tetrapods (crown-group plus all fossil taxa inside the crown) than to extant lungfishes (Clack, 2002; Coates *et al.,* 2008). In this thesis, we use a narrow, trait-based definition of the tetrapod group, wherein Tetrapoda is the clade containing those vertebrates which have paired limbs rather than paired fins (Goodrich, 1930). The origin of the tetrapods was the last of the evolutionary transitions that involved the emergence of a radical new body plan for animals (Shubin *et al.,* 2006). The taxa that spanned this transition encompassed 12-15 groups, the interrelationships of which is highly disputed, ranging from the Late Devonian to the Middle Cretaceous, some 380 to 100 million years ago (Coates *et al.,* 2008). The early tetrapods are extremely widespread across the globe and have been discovered in numerous localities (Clack *et al.,* 2016; Porro *et al.,* 2015; Thulborn *et al.,* 1996).

Important new early tetrapod discoveries over the past few years have reinvigorated interest in the study of the groups relationships. Before then fossil material from the transition were scarce with knowledge mainly being based upon informed guesswork (Clack, 2012). Tetrapods such as those of the Devonian have added considerable new comparative morphological data; before the 1990s only three taxa from the Devonian were known (Benton, 2015). Professor Jenny Clack (University of Cambridge) and other UK scientists improved our knowledge of early tetrapods vastly by conducting field expeditions that yielded many new specimens (Ahlberg, 1995; Coates & Clack, 1990; Schoch, 2014). Included amongst new finds was excellently preserved *Acanthostga gunnari* (fig. 1) material (Bendix-Almgreen *et al.,* 1990; Clack, 2002; Coates & Clack, 1991). Previously poorly known, *Acanthostega* was found to be a transitionary animal displaying both tetrapod and fish characteristics. The discovery of this animal was extremely important as it showed that the first tetrapods were in fact aquatic animals rather than being fully terrestrial (Coates & Clack, 1991). This indicates that terrestrialisation in tetrapods is made up of two different events, the origination of limbs from fins and the colonisation of land. These two events occurred separately with the colonisation of land being thought to have occurred in the Carboniferous at around 350-360 million years ago (Clack et al., 2016). The fin-limb transition is thought to have occurred earlier, during the Late Devonian at around 370 million years ago (Clack, 2012). Both of these events occurred in the Palaeozoic, an era in which changes occurred to the early tetrapod body plan which shaped terrestrial life as we know it.

1.1 Palaeozoic Early Tetrapods

The Palaeozoic is the earliest era of the Phanerozoic, the Eon in which vertebrates and many other groups originate (Hedges *et al.,* 2015; Kumar & Hedges, 1998). The Palaeozoic is split into six periods: Cambrian, Ordovician, Silurian, Devonian, Carboniferous and Permian. The first period in which tetrapods are found is during the Devonian. The Devonian (419mya-358mya), aptly named the age of fishes, was vastly different to the present day with lower

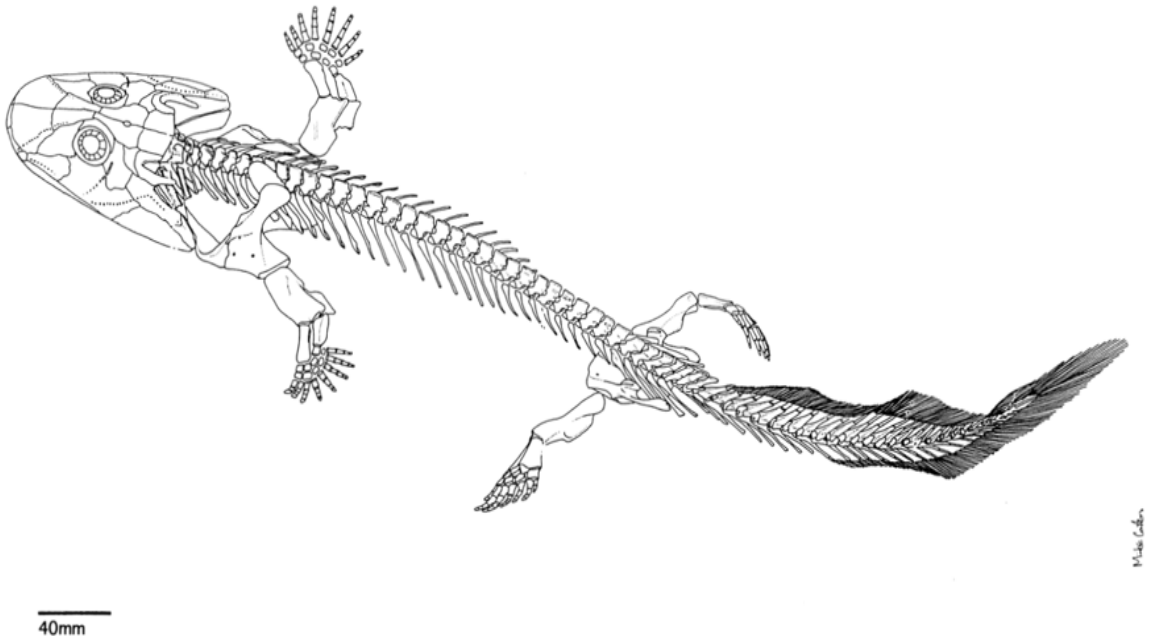


Fig 1. A skeletal reconstruction of *Acanthostega gunnari* (stem-tetrapod) from Coates, 1996. This reconstruction shows the fish-like aspects of the earliest early tetrapods, including fin rays on the tail and increased digits on the limbs.

oxygen levels, higher temperatures and different continents (Clack, 2012). The tetrapods found in the Devonian are thought to occupy the stem of the tetrapod group, before the crown (Ruta & Coates, 2007). However, there is controversy as to which specimen is considered the first tetrapod. Studies originally pointed at *Kenichthys*, a sarcopterygian fish from the Middle Devonian being the first (Coates *et al.,* 2002; Ruta & Coates, 2008). Recent studies documenting new tetrapod discoveries, however, have the potential to push the earliest known tetrapod back to the Lower Devonian (Lu *et al.,* 2012).

The most basal of the taxa from the Devonian are transitional forms between fish and tetrapods such as *Panderichthys* and *Tiktaalik* (Ahlberg & Clack, 2006). These more transitional forms had paired fins rather than limbs but did have many tetrapod features such as, a reduced dermal

exoskeleton in *Tiktaalik* and dorsally placed orbits in *Panderichthys* (Ahlberg & Milner, 1994; Shubin *et al.,* 2006). Currently, the earliest tetrapods with digits discovered were found during the Fransian stage of the Late Devonian. Tetrapods from the Devonian with digit- bearing limbs are only known in three genera: *Acanthostega*, *Ichthyostega* and *Tulerpeton* (Coates *et al.,* 2008). Other species in the Devonian may have had digits but they have simply not been discovered yet (such as *Elginerpeton* (Clack, 2012)). Even though the taxa had many 'terrestrial' traits tetrapods in the Palaeozoic were thought to have spent most of their time in water. *Ichthyostega* for example, is thought to have been an aquatic animal able to hold its head out of the water to breathe and potentially feed (Pierce *et al.,* 2012).

At the end of the Devonian to the Mid-Mississippian during the Carboniferous there is a period of time called Romer's Gap, which up until recently had an almost non-existent record of early tetrapod taxa (Smithson *et al.,* 2012). Many theories tried to explain this gap, from low oxygen levels causing unfavourable conditions (Ward *et al.,* 2006) to a bias in sampling methods meaning fossils simply have not been found yet (Smithson *et al.,* 2012). Recently, five new species of early tetrapod have been discovered during this period essentially closing the gap (Clack *et al.,* 2016). The study also showed that during Romer's gap oxygen levels stayed constant and normal from what they were during the Devonian, showing that the lack of fossils from that time was due to collection failure rather than environmental reasons. This shows how new discoveries can impact early tetrapod phylogeny greatly.

Moving towards the end of the Palaeozoic through the Carboniferous (358mya- 298mya) and Permian (298mya- 251mya) early tetrapods undergo many changes. The Carboniferous was the age in which tetrapods fully began to colonize the land. Due to this the tetrapod body plan became far more efficient for terrestrial life with, for example, a reduced number of digits and an ear that could capture sound energy travelling through air (Clack, 2012). During the Carboniferous, early tetrapods split into two groups, the stem-amphibians (mostly, the temnospondyls) and the stem-amniotes (anthracosaurs and their allies). Temnospondyls are the

most numerous and diverse group of early tetrapods, comprised of both fully aquatic and fully terrestrial taxa. They possessed four digits, large openings in the palate and short, straight ribs (Schoch & Milner, 2014). The stem-amniotes have a slightly different body plan with less flattened skulls, curved ribs, more complex and robust shoulder and pelvic girdles (Clack, 2012). The timing of the splitting event between the two groups continues to change, with new discoveries of early tetrapods and increased knowledge into previously discovered taxa offering potential for refined estimates (Clack *et al.,* 2016).

Early tetrapod phylogeny is still quite unstable, with new discoveries comes new reshuffling of groups (Ahlberg & Clack 1998; Ahlberg *et al.,* 2000; Sookias *et al*., 2014). There have been various previous studies that aim to reconstruct the early phase of tetrapod evolution (Bolt & Lombard, 2001; Clack & Ahlberg, 2004; Clack *et al.,* 2012; Ruta & Clack, 2006; Ruta & Coates, 2007) the most notable being Ruta & Coates (2007), which aimed to create a large dataset of osteological characters for the whole skeleton for a large number of early tetrapod taxa. Many of the studies focus on the skull or complexes within the skull (Ruta & Bolt, 2008). However, despite much progress in this area, there is as yet no overarching, comprehensive treatment of early tetrapod evolution that takes into account the entire database of characters for the cranium. This project aims to rectify this by creating the largest database of early tetrapod skull characters to date, including as many characters as possible for the whole skull. An example of an early tetrapod skull, and the elements that form the skull are shown in fig 2.

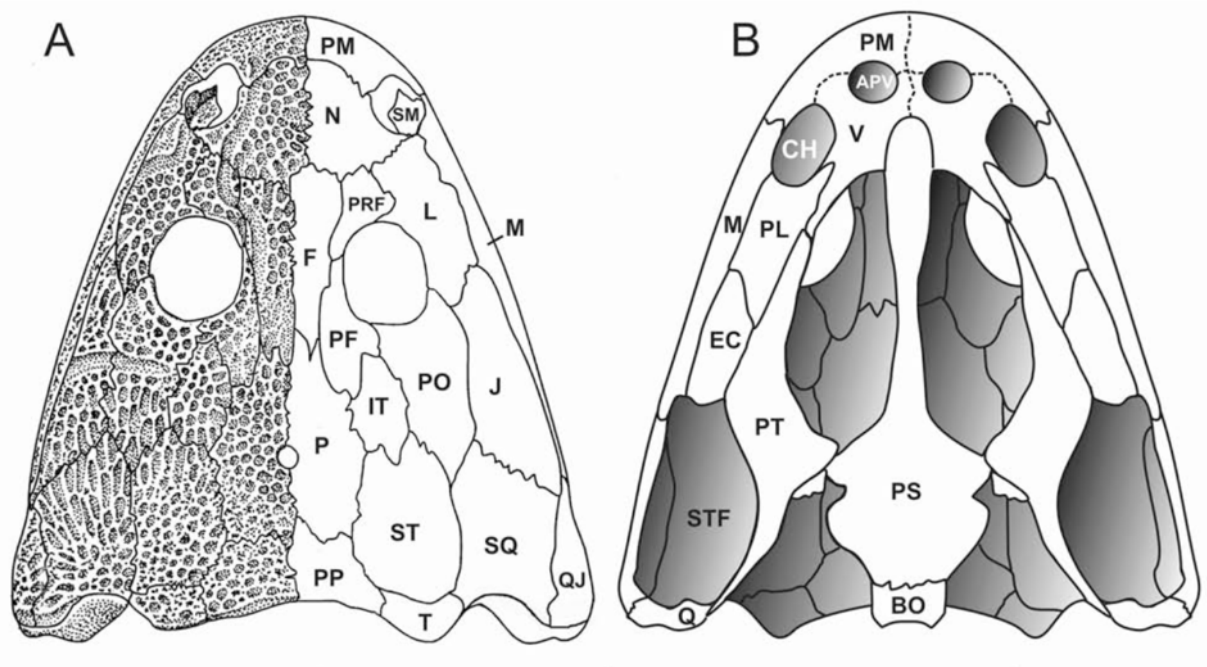


Fig 2. A reconstruction of the skull of *Trimerorhachis insignis* (stem- amphibian) from Milner & Schoch, 2013. This reconstruction shows the different complexes that make up an early tetrapod skull table (A) and palate (B): PM- premaxilla, N- Nasal, SM- septomaxilla, PRF- prefrontal, F- frontal, L- lacrimal, M- maxilla, PF- postfrontal, PO- postorbital, J- jugal, IT- intertemporal, P- parietal, PP-postparietal, ST- supratemporal, SQ- squamosal, QJ- quadrojugal, T- tabular, APV- anterior palatal vacuity, V- vomer, CH- choana, PL- palatal, EC- ectopterygoid, PT- pterygoid, Q- quadrate, PS- parasphenoid, BO- basioccipital.

1.2 Aims

The first overarching aim of this project is to synthesise all databases of skull characters available, with an aim to reject or amend poorly formulated characters and introduce new ones. Many skull complexes in early tetrapods are poorly represented in published character matrices, and this is particularly evident in the case of the braincase and palate (Clack *et al.,* 2012; Clack & Finney, 2005). Following the assembly of the largest and most comprehensive database for Palaeozoic tetrapod skull characters to date this project seeks to investigate disparity amongst

taxa, aiming to shed light upon how tetrapod craniums are placed within morphospace. This will be undertaken using a time calibrated version of the best fitting tree according to the parsimony criterion. An analysis looking at disparity in Palaeozoic early tetrapod skulls has not been undertaken before, we aim to rectify this and explore disparity both in morphospace and throughout time. Lastly, the rate of character state transformations across the tree will be assessed in the interest of unravelling the pattern of evolution in early tetrapods. Analyses of rate have been undertaken in early tetrapods but none focus specifically on the skull or have a database as large as the one used here (Anderson *et al.,* 2013; Ruta *et al.,* 2006). Rate will be investigated looking at both changes on specific branches and on the tree as a whole. The completion of these aims will give us a new perspective on Palaeozoic early tetrapod evolution and can be used as a starting point for future studies.

Therefore, my Masters project has been designed to contain three main objectives that when combined guarantee achievement of my core aim: Objective 1 focused on the creation of a new dataset that will lead to a new and expanded phylogeny for early tetrapods (using cranial characters), which in turn allows Objectives 2 and 3 to be achievable. My first core question (Objective 2) investigates whether the process of phylogenetic diversification (i.e., evolutionary branching) from the stem ancestor of tetrapods led to the origin of three main clades (stem-tetrapods, stem-amphibians and stem-amniotes), which evolved distinctive phenotypic patterns across clades, but which cluster within each individual clade. I hypothesise that phylogenetic diversification resulted in higher degrees of morphological disparity among clades relative to the degrees of within clade variation. My second core question (Objective 3) investigates whether the diversification rates of tetrapods followed a process consistent with an adaptive radiation where early bursts of diversification are followed by a slowing down as niche space gets filled by accumulation of lineages. I hypothesise that the diversification of early tetrapods is consistent with a process of niche-filling diversification (i.e., adaptive radiation).

2. Phylogenetic Analysis of the Palaeozoic Early Tetrapod Radiation: a novel step forwards toward a new phylogeny

Abstract

Early tetrapods are a group that cover a major evolutionary transition, the transition from water to land. Our knowledge of the group has improved tremendously over the past few years, with new discoveries comes a new picture of the early tetrapod phylogenetic tree. This study aimed to create the most comprehensive database of cranial characters for early tetrapods to date. This database was used to create a new tree for the group, using taxa that cover the transition in question closely. Parsimony analysis was employed to find the best fitting tree from our data. We retrieved a well resolved tree that shows a pattern of species the same, for the most part, as previous studies. Our analysis pushes back the divergence between the stem-amphibians and the stem-amniotes to the Upper Devonian, earlier than previous estimates. The inclusion of the new taxa (*Koilops, Diploradus, Aytonerpeton* and *Perittodus*) are the main reason for this new placement of the split. Future study with an expanded character list (including postcranial data) would be the next step in further understanding relationships between early tetrapods in the Palaeozoic.

2.1 Introduction

Due to the nature of the fossil record early tetrapod phylogeny has gone through many different iterations with much debate surrounding the subject. Early tetrapod evolutionary trees have evolved greatly over the past few years, with the wealth of discoveries since the 1980s closing many gaps in the groups phylogeny (Ahlberg, 1995; Clack, 2002; Coates & Clack, 1991). Recent study into Palaeozoic early tetrapod relationships has indicated that the groups phylogeny may be starting to stabilize due to similar branching patterns arising (Clack *et al.*, 2012(2); Ruta & Coates, 2007; Sookias *et al.,* 2014). An extensive analysis carried out by Ruta

& Coates (2007) for example, places most Carboniferous limbed tetrapods within the crown, whereas all Devonian taxa are excluded from the crown. However, this pattern is now changing dramatically with studies pointing at a different pattern of interspersed Devonian and Carboniferous taxa (Clack *et al.,* 2016).

Due to the discovery of new species, as well as improved knowledge of existing species, early tetrapod phylogeny has been prone to reshuffling (Sookias *et al.,* 2014; Clack *et al.*, 2012(2); Friedman *et al.,* 2003). For example, recent discoveries in the Scottish Borders of five new Tournaisian tetrapods has caused a rethink into the placement of the amphibian-amniote split, the event when the two lineages split apart as shown in Clack *et al.,* 2016. The results of this study indicate that there is a substantial reshuffling of Carboniferous stem-tetrapods, with the placement of some of the new taxa amongst stem-amphibians and amniotes potentially pushing the split backwards. The new discoveries of early tetrapods are beginning to reshape our understanding of tetrapod evolution, informing novel patterns of relationships, challenging some long-standing hypotheses, and resulting in a more elaborate construction for the stem-group.

This thesis focuses upon creating the most extensive database for cranial characters to date. Cranial characters are being used in isolation for a number of reasons. Firstly, the skull is easy to atomise compared to the postcranium with many complexes that can be easily characterised. The cranium is far easier to divide up into separate traits than the postcranium. For example, the shape and length of the ribs could be used as a character, however, distinguishing between one state and the other or where one trait should start and another should end could prove difficult. Secondly, the skull is a feature more widely studied than the postcranium which has not been subjected to detailed scrutiny (Clack & Milner, 2015; Schoch & Milner, 2014; Ahlberg & Clack, 1998). Lastly, the majority of characteristics that change during the transition from fish to tetrapods are unsurprisingly concerned with locomotion. However, some of the of the

most important changes are concerned with the skull due to the transformations associated with feeding and breathing (Clack, 2012).

The Palaeozoic early tetrapod skull is made up of a number of different elements, the number of which are mainly consistent amongst the group as a whole. The more primitive members of the group however, do show some differences in general composition of the skull, with a mixture of both tetrapod and fish characteristics. For example, Early Devonian tetrapod *Tiktaalik* has tetrapod-like features such as the loss of the opercular series of bones which covered the gills (Clack & Milner, 2015) and fish-like features such as a short, straight hyomandibular (Downs *et al.,* 2008). There are also many skull features that uniquely identify certain groups, for example temnospondyls have large openings in the palate, a strut-like and elongate ventral portion of the braincase, broad and flat vomers, among other features (Schoch & Milner, 2014).

This chapter presents a cladogram for Palaeozoic early tetrapods using the full suite of skull characters available and a wide coverage of species from the fin-limb transition. Due to frequent new discoveries, it is important to continually revaluate relationships between species in order to fully understand the early tetrapod group as a whole. In order to infer relationships, we chose parsimony as the optimality criterion. According to this criterion, the most likely tree is the one that requires the fewest instances of character state transformations (Hall, 2011). Parsimony analysis operates by selecting a tree or multiple trees that have the fewest number of evolutionary steps required to explain the data.

The aims of this chapter are to:

i. Build a near-comprehensive database for Palaeozoic early tetrapod cranial characters, the largest assembled to date, that will be used to discuss hypotheses of relationships among the major groups of early tetrapods.

ii. Assess statistical support for branching patterns. Support will be evaluated for key branching episodes, such as the fish-tetrapod transition and the separation among major clades.

iii. Assess the impact of new taxa upon the branching pattern. We hypothesise that the four new taxa (*Koilops, Diploradus, Perittodus* and *Aytonerpeton*) will impact the branching pattern, potentially pushing the amphibian-amniote split to an earlier position in time (compared to previous studies).

iv. Create a time calibrated tree for use in subsequent analyses, where models of evolution are explored.

2.2 Method

2.2.1 *Data Matrix*

A data matrix consisting of 35 taxa coded for 347 cranial characters was built in Mesquite (Maddison & Maddison, 2016). The characters were compiled from previous datasets (Bolt & Lombard, 2001; Clack & Ahlberg, 2004; Clack *et al.,* 2012(2); Ruta & Clack, 2006; Ruta & Coates, 2007; Ruta & Bolt, 2008) and were vetted and checked for mistakes in addition to areas of uncertainty. Each character includes a description of the relevant trait of interest and a selection of alternative conditions of that trait (see Appendix 2 for character list). For instance, the following example refers to a bone occupying the anterior end of the dorsal aspect of the lower jaw. This bone, termed the adsymphysial, can be coded for its occurrence or complete absence across different species: ADSYM 1- Presence (0) or absence (1) of adsymphysial plate. Preceding the character explanation is an identifier (e.g. ADSYM) and a number that allows easy retrieval of the character in the assembled list. This allows simple character removal or addition that will not affect the coding of any other character in the dataset. Areas of uncertainty

or complete absences of certain structures due to incomplete specimens are coded as a question mark.

When choosing characters from the literature many of them had slightly different wording or character states. With characters with different wording, we chose the character we felt was the most unambiguous and explained the character in the best way. For example, Ruta & Coates (2007) describes a quadratojugal character as: QUAJUG 2 - Quadratojugal depth less than one-fourth of squamosal depth: absent (0); present (1). While in Ruta & Clack (2006) the size of the Quadratojugal compared to the squamosal is simply quantified as: QUAJUG 2 - Absence (0) or presence (1) of condition: quadratojugal much smaller than squamosal. We decided to use the Ruta & Coates character as the size comparison of the two components (bones) can be more easily quantified.

When a mismatch in coding was found between two matrixes we referred back to the source material (images/ illustrations of complexes) to find the best coding possible. Usually difference in coding with our own or between two other matrices is due to an obvious mistake. Where reason for a certain coding was more ambiguous we applied our own interpretation of the character and made sure to be consistent throughout coding for all 35 taxa. When one author coded a state while another coded as a question mark, the later was usually due to incomplete specimens at the time of characterisation. Due to this the coded character is used, after checking on the source material.

This project will focus on taxa that bracket closely the fin to limb transition as well as taxa that span the stems of the two major tetrapod radiations – the amphibians and the amniotes. Later groups are beyond the scope of this thesis. Chosen taxa were included if complete or partially complete cranial material was available. The taxa used (with the specimen ID numbers of the main specimens used) are: *Acanthostega gunnari* (MGUH-VP-8160, MGUH-VP-8158 & MGUH 29019); *Archeria crassidisca* (MCZ 2063, MCZ 2121 & MCZ 2052); *Aytonerpeton*

microps (UMZC 2015.46); *Balanerpeton woodi* (GLAHM V2051, UMZC T1312 & BMNH R12014); *Caerorhachis bairdi* (MCZ 2271); *Cochleosaurus bohemicus* (MB Am 80, NMS G 1898/105/28/29); *Cochleosaurus florensis* (MCZ 4343, MCZ 4344 & MCZ 4342); *Crassigyrinus scoticus* (GSE 4722 & BMNH R10000); *Densignathus rowei* (ANSP 20637.1 & ANSP 20637.2); *Diploradus austiumensis* (UMZC 2015.30); *Disosauriscus astriacus* (K13, K40, K313 & KO 64); *Doragnathus woodi* (NUZ 77.5.26 & RSM GY 1898.107.51); *Edops sp* (MCZ 1378 & MCZ 1235); *Elginerpeton pancheni* (GSM 89174, BMNH P63658 & LEICS G612.1993); *Eryops megalocephalus* (AMNH 4189 & AMNH 4556); *Gephyrostegus bohemicus* (UMZC T107, UMZC T1337 & NMP M885); *Greererpeton burkemorani* (KUVP 126315, KUVP 126314 & CMNH 10931); *Ichthyostega stensioei* (MGUH VP 6057a, 6138, 6163 & MGUH 28376); *Koilops herma* (NMS G. 2013.39/14); *Megalocephalus pachycephalus* (HM G 15.21, HM G 15.46, BMNH R2363 & BMNH R585); *Metaxygnathus denticulus* (ANU 28780A-B); *Obruchevichthys gracilis* (PIN 1491/52); *Occidens portlocki* (GSM 28498); *Onchiodon labrinthicus* (UMZC T.117); *Panderichthys rhombolepis* (LDM 43/293, LDM 257/1399 & PIN 3547/18); *Perritodus apsconditus* (UMZC 2011.7.2a & UMZC 2016.1); *Pholiderpeton scutigerum* (MCZ 6976 & MCZ 6977); *Proterogyrinus scheelei* (MCZ 4537, USNM 22573 & CMNH 11067); *Sigournea multidentata* (FM PR 1820); *Spathicephalus mirus* (NMS G 1950.56.3, 1950.56.4 & NMS G 1885.50.73); *Tiktaalik roseae* (NUFV 108 & NUFV 116); *Trimerorhachis insignis* (AMNH 4565); *Ventastega curonica* (LDM G 81/775, LDM G 81/776 & LDM G 81/777); *Whatcheeria deltae* (FM PR 1643 & FM PR 1700); *Ymeria denticulate* (MGUH VP 6088 & MGUH VP 6026). See Appendix 1.2 for references and institution list.

Unlike previous analyses, the present study is novel in combining taxa that either were not used previously or were used in very few studies with a limited sampling of characters (e.g. *Ymeria* (Clack *et al.,* 2012(1)). As well as characters that have been atomized in some previous studies, such as lower jaw traits, but not included in full alongside other character sets (Ruta & Bolt, 2008). Coding of characters was carried out via the use of literature outlined in the character

Species	Locality	FAD	LAD
Acanthostega gunnari	Gauss Halvø, East Greenland	376.1	360.7
Archeria crassidisca	Texas, USA	296.4	279.5
Aytonerpeton microps	Berwickshire, Scotland	360.7	345.3
Balanerpeton woodi	West Lothian, Scotland	336	326.4
Caerorhachis bairdi	Midlothian, Scotland	326.4	318.1
Cochleosaurus bohemicus	Nyrany, Czech Republic	311.45	306.9
Cochleosaurus florensis	Nova Scotia, Canada	311.45	306.9
Crassigyrinus scoticus	Fife, Scotland	326.4	318.1
Densignathus rowei	Pennsylvania, USA	364.7	360.7
Diploradus austiumensis	Berwickshire, Scotland	360.7	345.3
Disosauriscus astriacus	Boskovice Furrow, Czech Republic	298.9	290.1
Doragnathus woodi	Cowdenbeath, Scotland	326.4	318.1
Edops sp	Texas, USA	295	290.1
Elginerpeton pancheni	Morayshire, Scotland	379.5	376.1
Eryops megalocephalus	Texas, USA	303.4	272.5
Gephyrostegus bohemicus	Nyrany, Czech Republic	311.4	306.9
Greererpeton burkemorani	West Virginia, USA	326.4	318.1
Ichthyostega stensioei	Ymers Ø, East Greenland	376.1	360.7
Koilops herma	Berwickshire, Scotland	360.7	345.3
Megalocephalus pachycephalus	Northumberland, UK	318.1	306.9
Metaxygnathus denticulus	New South Wales, Australia	376.1	360.7
Obruchevichthys gracilis	Unknown, believed to be Russia	383.7	376.1
Occidens portlocki	Londonderry, Northern Ireland	360.7	326.4
Onchiodon labrinthicus	Saxony, Germany	298.9	290.1
Panderichthys rhombolepis	Zheleznogorsk, Russia	383.7	382.4
Perritodus apsconditus	Berwickshire, Scotland	360.7	345.3
Pholiderpeton scutigerum	Bradford, UK	314.6	311.4
Proterogyrinus scheelei	West Virginia, USA	326.4	318.1
Sigournea multidentata	Iowa, USA	339.4	336
Spathicephalus mirus	Midlothian, Scotland	326.4	318.1
Tiktaalik roseae	Nunavut, Canada	383.7	376.1
Trimerorhachis insignis	Texas, USA	290.1	272.5
Ventastega curonica	Saldus district, Latvia	376.1	360.7
Whatcheeria deltae	Iowa, USA	339.4	336
Ymeria denticulate	Kejser Franz Joseph Fjord, East Greenland.	376.1	360.7

Table 1. Species used in this study, locality of type species and first appearance (FAD) and last appearance (LAD) datums (in million years ago).

list and in first hand for a selection of taxa.

2.2.2 *Parsimony Analysis*

Cladistic analysis was carried out via PAUP* version 4.0a b154 (Swofford, 2003). Parsimony analysis operates by selecting a tree or multiple trees that have the fewest number of evolutionary steps required to explain the data. A heuristic search (TBR branch swapping, all characters unordered, 1000 replicates) was carried out with *Panderichthys rhombolepis* assigned as the outgroup (Schoch, 2014).

For each tree, descriptive statistics are reported. Firstly, the tree length shows the amount of character state transformations on a tree, the better the fit the less steps there are (Hall, 2011). The consistency index (CI), retention index (RI) and rescaled consistency index (RC) measure the fit of data to a tree. The consistency index measures the amount of homoplasy on a tree. This is calculated by the minimum number of changes divided by the number observed the tree, with the minimum number of changes for the character being equal to the number of its states minus one. The number of changes on the tree is the one accounted for by the phylogeny. CI works on a scale from 0 to 1. If the CI is 1 then there is no homoplasy recorded on the tree. Also, trees with a higher CI are seen as more being more reliable (Archie, 1989). The retention index (maximum steps (taxa with state 1 or 0) minus observed steps (CI) divided by the maximum steps minus minimum steps (CI)) shows the proportion of taxa that have states that are not homoplastic. The retention index is used to measure the amount of parsimony uninformative characters (Farris, 1989). Finally, the rescaled consistency index is simply the product of CI x RI.

A second heuristic search was carried out with the characters reweighted by the maximum value of their RI from the initial analysis. Weighting of characters simply acts on the notion that not all characters are of equal phylogenetic value and assigns each character a weight depending on how important they are. Finally, bootstrap analysis is carried out upon the chosen tree using

PAUP*. Bootstrap analysis is carried out by randomly replacing characters within the dataset, re-running the parsimony process and assessing the confidence of the analysis reflecting the real phylogeny (Efron *et al.,* 1996). This is process is repeated multiple times during the analysis and evaluates the support for the branching pattern retrieved; with support over 75 being deemed to be good, but below 75 is deemed as moderate or weak.

2.2.3 *Time Calibration*

The chosen most parsimonious tree was time calibrated in order to be used for future analyses. Time calibration of a cladogram adds the stratigraphic information of species to the tree, allowing it to be used in analyses of rates and disparity. First appearance data (FAD) and last appearance data (LAD) (table 1) were collated using the Fossilworks Palaeobiology Database (found at: http://fossilworks.org). Time calibration was carried out using the R language with the package Strap and the geoscalePhylo function. R outputs the same tree but with stretched branches pertaining to geological time.

2.3 Results

2.3.1 *Phylogenetic Analyses*

A heuristic search was carried out and 518 equally parsimonious trees with 884 steps were recovered (CI= 0.36, RI= 0.53, RC= 0.19). The strict and majority (50%) consensus' of these trees is shown in fig. 3. Characters were reweighted and a heuristic search was carried out retrieving 11 trees, the strict and majority (50%) consensus' for which are shown in fig. 4. The tree is well resolved apart from a polytomy consisting of 6 taxa at the top of the tree: *Ventastega, Tiktaalik, Ymeria, Obruchevichthys, Elginerpeton and Metaxygnathus.*

An agreement subtree was calculated in order to highlight if there were taxa that may be reducing signal. An agreement subtree is a pruned cladogram for which all shortest trees agree

upon relationships (Ruta & Coates, 2007). The agreement subtree consisted of 31 taxa. The Four pruned taxa were: *Elginerpeton, Obruchevichthys, Metaxygnathus* and *Ymeria*. Two taxa, *Obruchevichthys and Metaxygnathus* were removed from the dataset due to the highly fragmentary nature of their specimens meaning that many characters were coded as areas of uncertainty. *Obruchevichthys* is especially fragmented with the holotype only consisting of small pieces of the lower jaw, while *Metaxygnathus* is known only from one jaw specimen alone (Ahlberg & Clack, 1998). These taxa were included in the first place to assess whether a robust signal can be gleaned from a fragmentary specimen. Other fragmentary taxa, such as *Occidens portlocki,* are placed confidently on the tree in this analysis.

A heuristic search was rerun with the two taxa removed and recovered 16 equally parsimonious trees (CI= 0.36, RI= 0.53, RC= 0.19) with 875 steps. A strict and majority (50%) consensus for these trees is shown in fig. 5. Due to the removal of the two taxa the polytomy at the top of the tree is now well resolved. Reweighting of characters was carried out using the 16 trees, recovering 1 tree (CI= 0.58, RI= 0.72, RC= 0.42) with 171 steps shown in fig. 6. This tree shows good resolution with no polytomies retrieved.

Bootstrap support was carried out (100 bootstrap replicates) using the 50% majority consensus of retrieved trees (fig. 7). High support (98%) for the grouping of the two species of *Cochleosaurus* and for the grouping of *Eryops* and *Onchiodon* (93%). The clade consisting of *Gephryostegus, Proterogyrinus, Archeria* and *Pholiderpeton* has bootstrap support of 71% with the grouping of the latter three having 85% support. Other tree branches have bootstrap support of less than 50%. The high bootstrap support for the grouping of *Cochleosaurus florensis* and *Cochleosaurus bohemicus* is not surprising as they are two species are rather similar (Rieppel, 1980). The high support for *Eryops megacephalus* and *Onchiodon labyrinthicus* is also not surprising given the numerous shared derived features of their family, Eryopidae, including circular, small, and widely spaced orbits, foreshortened skull table, and broad snouts (Clack & Milner, 2012).

The taxa have been placed in these groups according to this analysis:

Stem-tetrapod

Panderichthys rhombolepis, Tiktaalik roseae, Ventastega curonica, Elginerpeton pancheni, Perritodus apsconditus, Densignathus rowei, Ymeria denticulate, Acanthostega gunnari, Ichthyostega stensioei, Aytonerpeton microps, Crassigyrinus scoticus, Occidens portlocki, Whatcheeria deltae, Greererpeton burkemorani, Diploradus austiumensis, Sigournea multidentata, Megalocephalus pachycephalus, Spathicephalus mirus and *Doragnathus woodi.*

Stem-amphibian

Edops sp., Eryops megalocephalus, Onchiodon labrinthicus, Cochleosaurus bohemicus, Cochleosaurus florensis, Balanerpeton woodi, Koilops herma and *Trimerorhachis insignis.*

Stem-amniotes

Caerorhachis bairdi, Disosauriscus astriacus Gephyrostegus bohemicus, Proterogyrinus scheelei, Archeria crassidisca and *Pholiderpeton scutigerum.*

The arrangement of taxa in our study shows similarity to other reports. For example, the tree from Pardo *et al* (2017) shows the same arrangement for the early stem, from *Panderichthys* to *Crassigyrinus*. The later portion of the tree shows a slightly different pattern to ours, but it can be noted that they use a largely different group of taxa than our study. The methods used by Pardo *et al* use a similar methodology to our own with a similar size of character list. However, the character list is slightly different to the one used here due to the use of taxa further removed from the transition. Due to this the pattern would have been expected to be different, especially with the taxa placed towards the crown. An analysis by Clack *et al.* (2012) also produced a tree with stem-tetrapods that also retrieved a similar branching pattern to our tree. Again, a different taxa set was used, however, a large part of the placement of taxa within the stem match with our reports.

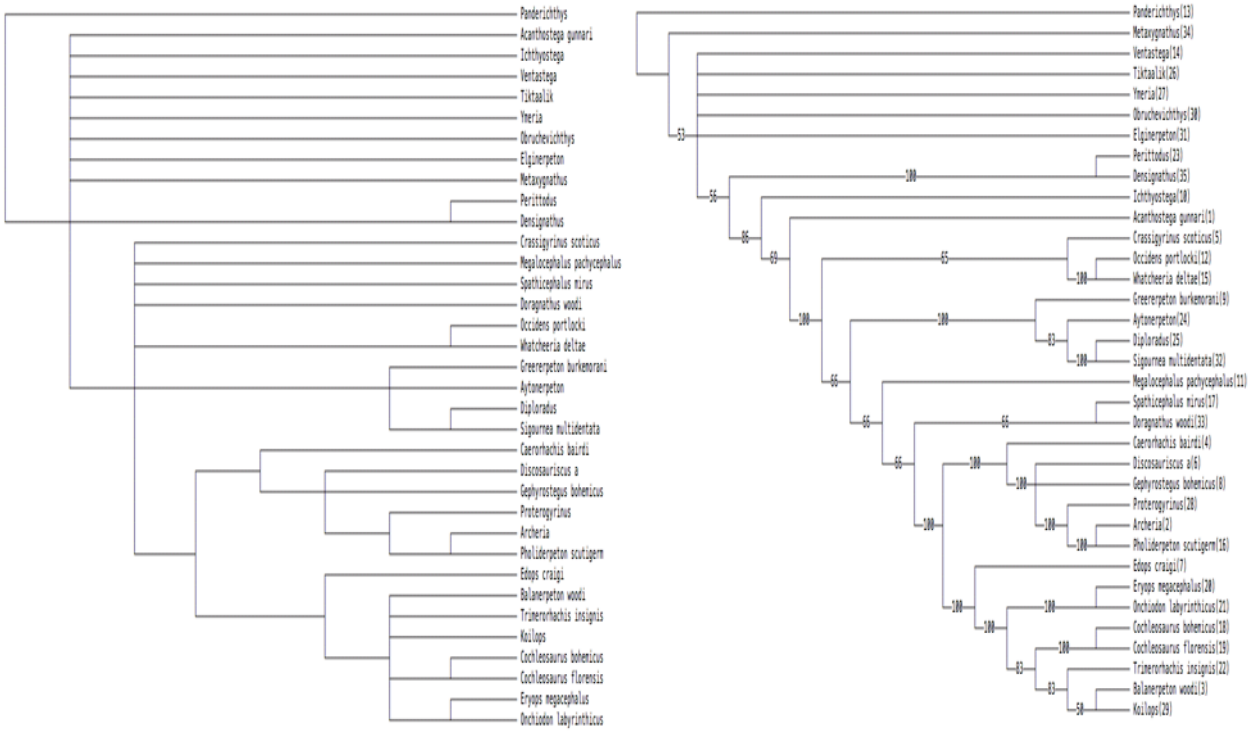


Fig. 3. (a) Strict and (b) majority consensus (50%). Numbers on majority consensus branches indicates percentage of the trees that retrieve that branching pattern (numbers next to species names are simply order of species in original dataset).

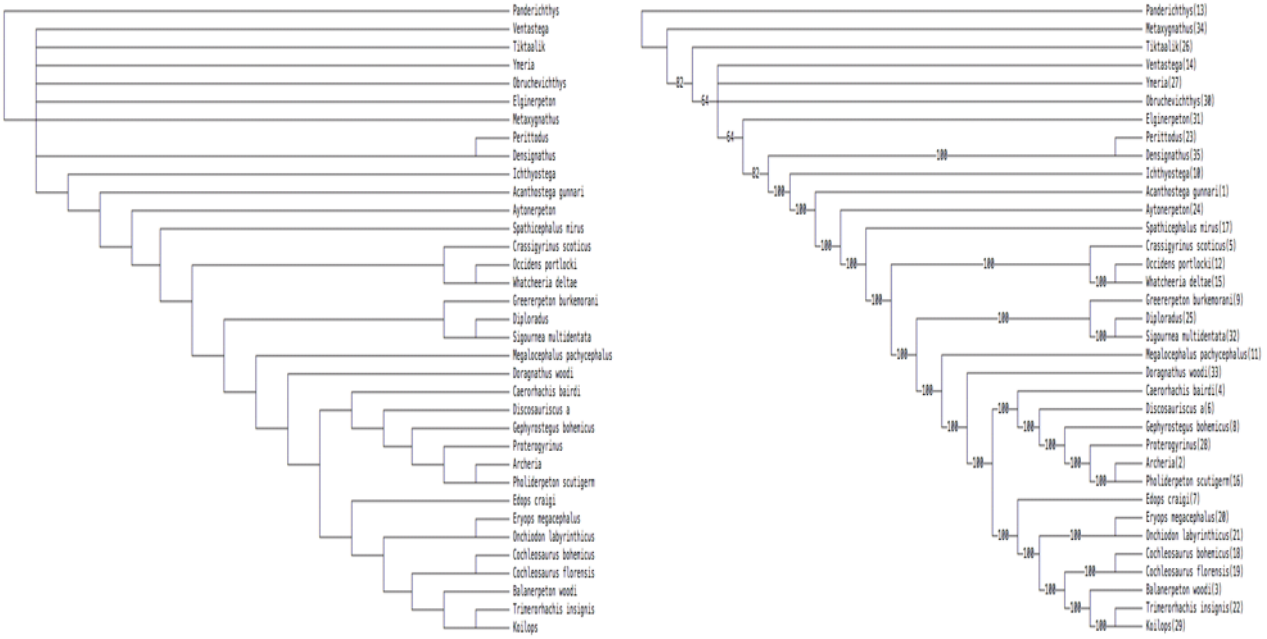


Fig. 4. (a) Reweighted strict consensus and (b) reweighted majority consensus (50%). Numbers on majority consensus indicates percentage of the trees that retrieve that branching pattern (numbers next to species names are simply order of species in original dataset).

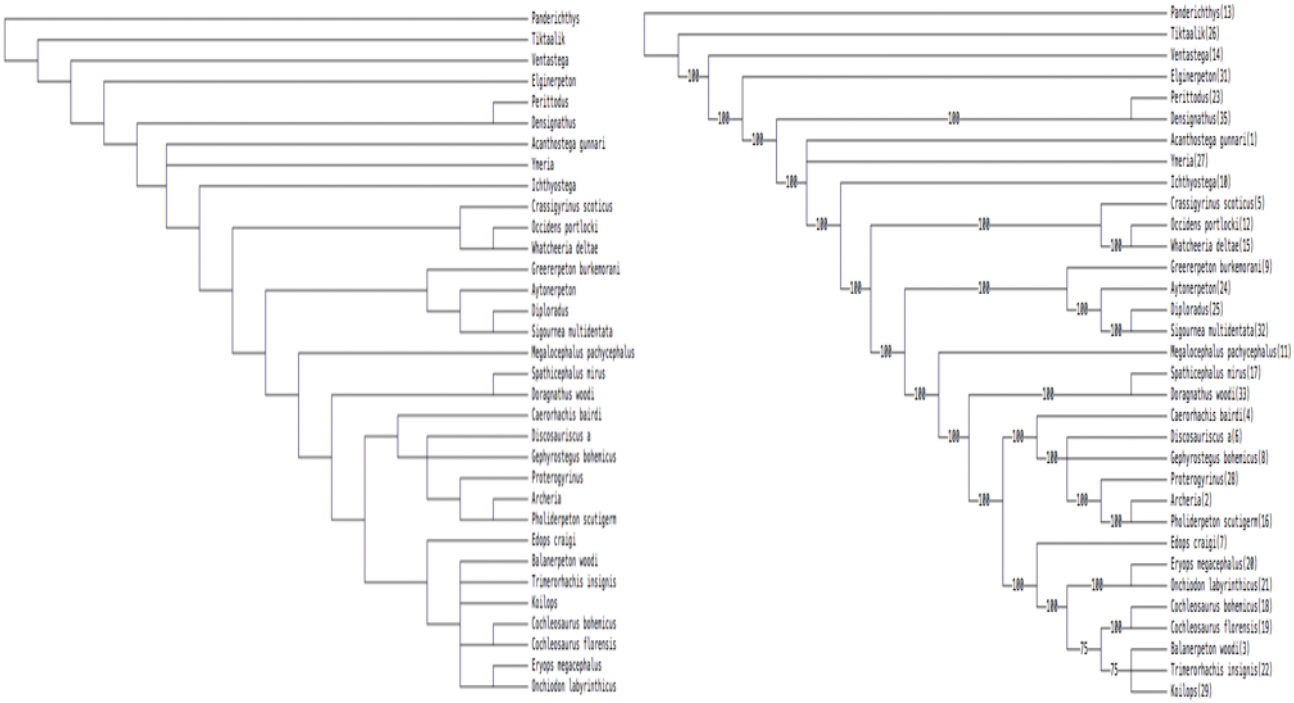


Fig. 5. (a) Strict consensus and (b) majority consensus (50%) with *Obruchevichthys* and *Metaxygnathus* removed. Numbers on majority consensus indicates percentage of the trees that retrieve that branching pattern (numbers next to species names are simply order of species in original dataset).

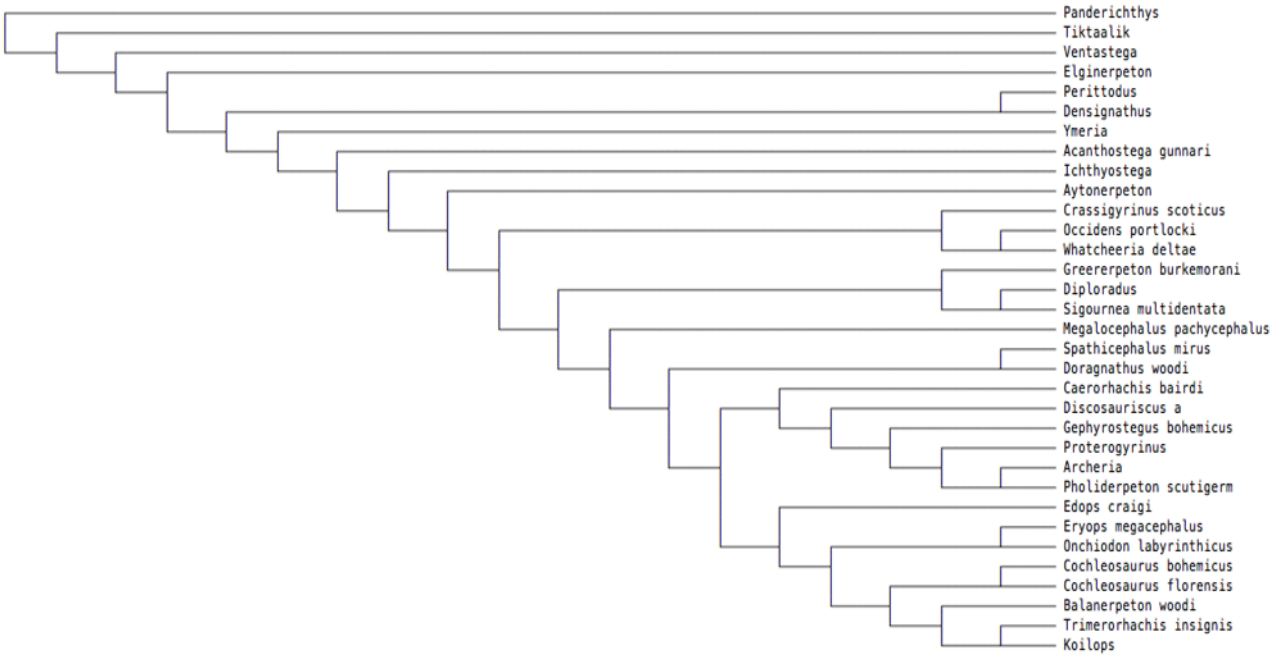


Fig. 6. Reweighted strict consensus with *Obruchevichthys* and *Metaxygnathus* removed.

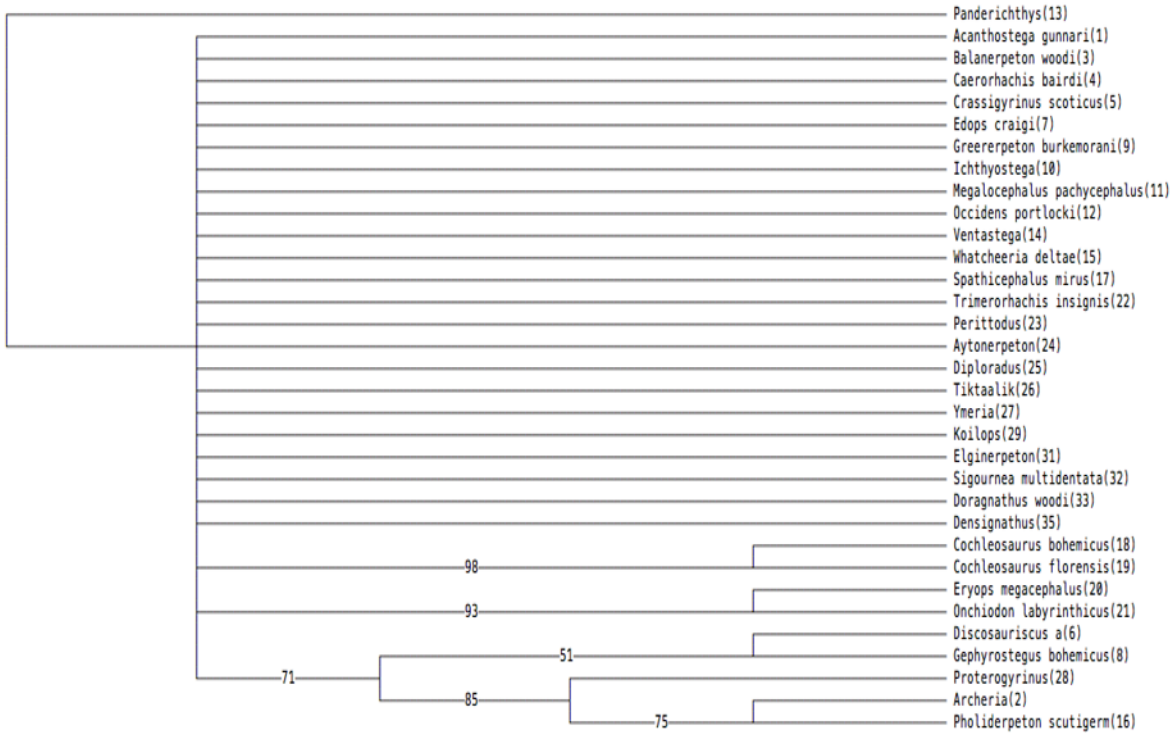


Fig.7. Bootsrap support for branching pattern. Numbers indicate bootstrap support for branches, absence of numbers indicate a bootstrap support of less than 50% (numbers next to species names are simply order of species in original dataset).

2.3.2 *Time Calibration*

The time calibrated tree is shown in fig. 8, this new calibration supersedes previous ones due to how comprehensive the dataset used to build it is. The amphibian-amniote split has been pushed back by 18 myr compared to other analyses (Hedges *et al.,* 2015; Kumar & Hedges, 1998) to the Famennian of the Upper Devonian, around 370 mya. This is due to the inclusion of the four new taxa, especially the placement of *Koilops* within the stem-amphibians. With the removal of *Koilops* the divergence is at around the same place as previous estimations, during the Early Carboniferous (fig. 9). This push back of the splitting event has effected divergence times of other events including pushing the timing of the fish-tetrapod transition to early in the Devonian. Some of the branches are extremely stretched for example the branch for *Trimerorhachis*. From this it could be inferred that there could be a lot of unknown diversity within this branch.

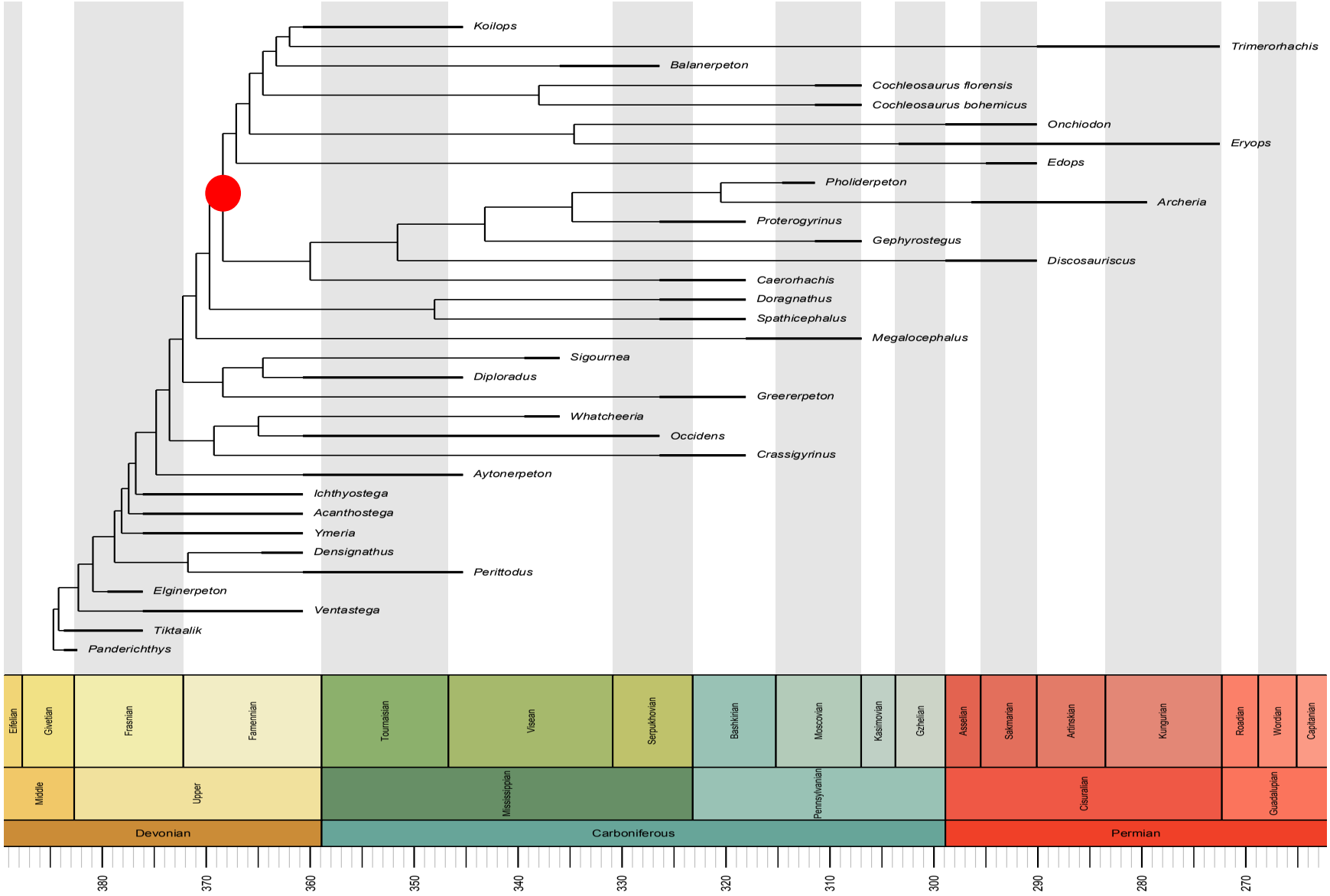


Fig. 8. Time calibrated tree. Thicker black lines indicate the currently observed time range for a species. The red dot indicates the branching point between the stem-amphibians and stem-amniotes.

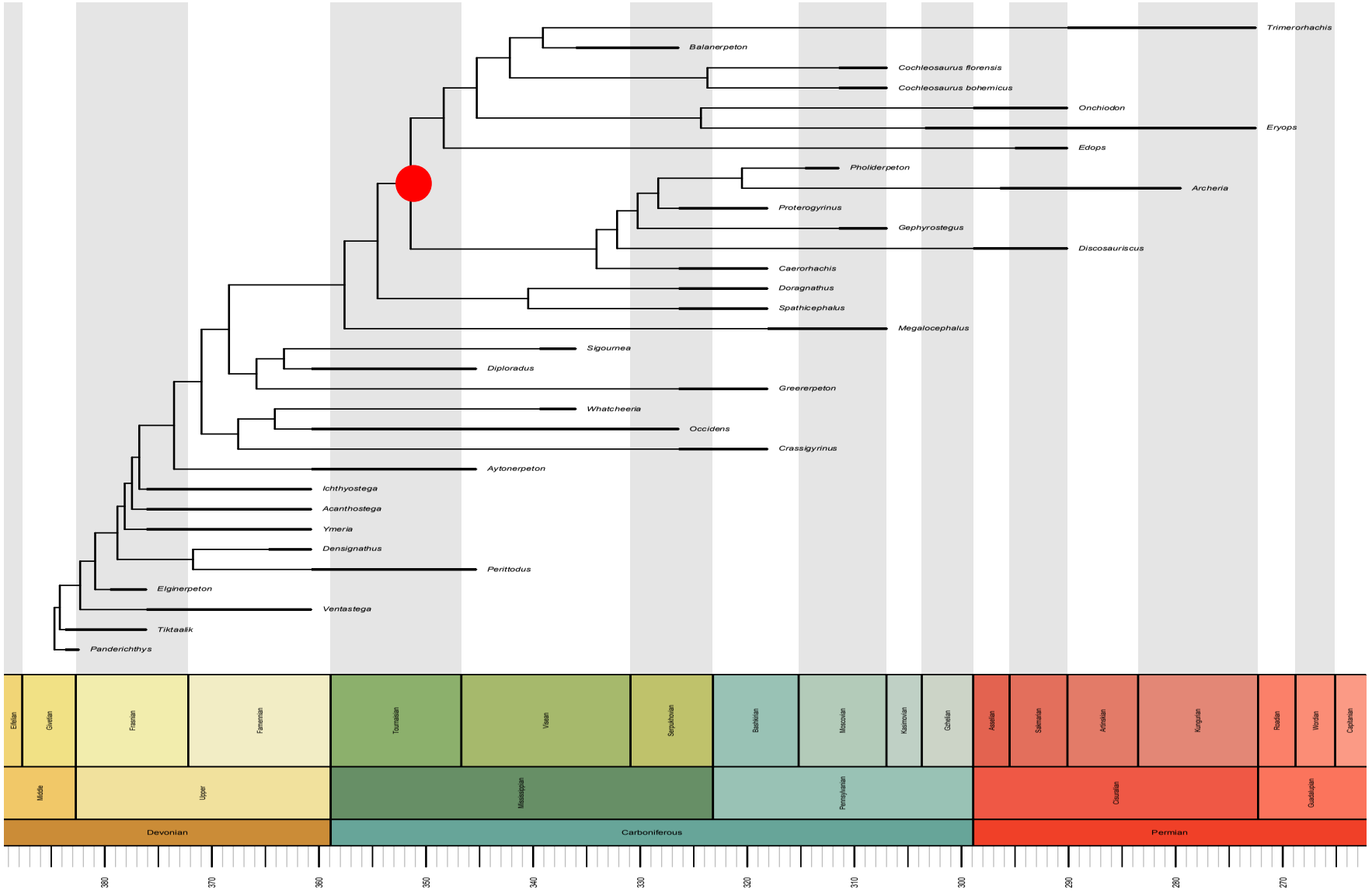


Fig. 9. Time calibrated tree excluding *Koilops.* Thicker black lines indicate the currently observed time range for a species. The red dot indicates the branching point between the stem-amphibians and stem-amniotes.

3.4 Discussion

This study provides the most comprehensive dataset and phylogeny using early tetrapod cranial characters ever assembled via modern modelling methods. By compiling an unprecedented dataset, my results show a tree with a pattern similar to previous trees while also revealing that the new early tetrapods (*Koilops, Diploradus, Aytonerpeton* and *Perittodus*) do impact the tree greatly, pushing back the divergence (compared to previous estimates) between stem-amphibians and stem-amniotes by a predicted 18 myr.

Early tetrapod relationships are still quite unstable, with new discoveries comes new reshuffling of species. This chapter aimed to create a cladogram for early tetrapods in the Palaeozoic, using the most comprehensive dataset of cranial characters to date and also assess the impact of new species upon the branching pattern. The cladogram retrieved (fig. 6) is a well resolved tree showing branching patterns previously found for Palaeozoic tetrapods, with members of the stem-tetrapods grouping together, the temnospondyls being placed as a group and branching from the stem-amniotes (Ruta & Bolt, 2008; Ruta & Coates, 2007; Pardo et al., 2017). The temnospondyls show a slightly different pattern to that of Ruta *et al.,* (2007b) with *Eryops* and *Onchiodon* being retrieved above the other members of the temnospondyls used in this analysis. A recent analysis carried out by Pardo et al., (2017) produced a tree for early tetrapods with the same placement as our analysis for most of the early tetrapod stem group. This shows that early tetrapod groups, especially the stem, may be becoming more stable in structure. Both our study and Pardo *et al.* use a large database of cranial (albeit different) characters further cementing the common placement of taxa, especially within the early stem.

An analysis by Clack *et al.,* (2012) produced a tree with stem-tetrapods and retrieved a similar branching pattern to our tree. However, the species *Caerorhachis* is placed differently in our analysis, by being placed along with the stem-amniotes rather than within the stem-amphibians, close to *Balanerpeton*. Clack *et al.* (2012) including postcranial characters could explain the

opposing the placement of the species. *Caerorhachis* has been a difficult species to place due to it possessing a mixture of both stem-amniote and stem-amphibian features (Ruta *et al.,* 2001). For example, large openings in the palate would indicate that the animal would group closer to the temnospondyls (stem-amphibians) rather than the stem-amniotes.

The four of the newly discovered species from Scotland included in this analysis: *Diploradus, Koilops, Aytonerpeton* and *Perittodus* impacted the tree significantly. In the retrieved tree (fig. 6) *Koilops* is placed within the Temnospondyls (stem-amphibians), closely grouped with *Balanerpeton* and *Trimerorhachis*. Clack *et al.,* (2016) found one analysis in which *Koilops* was in fact placed within the stem-amphibians, however, other analyses place it as a stem-tetrapod. *Koilops* is quite a generalised animal which could be the reason it is placed close to the also quite generalised *Balanerpeton* in this analysis. The inclusion of more taxa in the dataset and the discovery of more complete *Koilops* specimens would vastly improve the placement of the species; it is currently known from one incomplete skull specimen alone. The placement of another of the new taxa, *Diploradus*, with *Sigournea* is interesting as they show some quite striking morphological similarities, especially in the shape of the lower jaw (Bolt & Lombard, 2006; Clack *et al.,* 2016). The placement of *Koilops* and *Perritodus* as a more basal stem-tetrapods backs up Clack's suggestion of a blurring of the Devonian/ Carboniferous boundary in respect of tetrapod evolution.

The placement of the new taxa also implies a deeper split between the stem-amphibians and stem-amniotes than previously thought (Hedges *et al.,* 2015; Kumar & Hedges, 1998; Reisz & Müller, 2004). This analysis places the split in the Upper Devonian, further back than previous estimates of the early Carboniferous (fig. 8). This is mainly due to the placement of *Koilops* amongst the stem-amphibians. The placement of *Koilops* in this analysis could be deemed as questionable due to the morphology of the species as well as it being a tetrapod from the early Carboniferous. However, as previously stated the Devonian/ Carboniferous boundary has been suggested to be more blurred than previous estimates (Clack *et al.,* 2016) so the analysis with

Koilops has been included in this study. When *Koilops* is removed (fig. 9) the placement of the split is pushed forward (from the placement in fig. 8) into the Carboniferous, at around the same point provided by Clack *et al.,* 2016 and the previous molecular dates (Hedges *et al.,* 2015; Kumar & Hedges, 1998).

In conclusion, the evolutionary tree of life of early tetrapods has been shown by these results to be a well resolved tree with a pattern of stem-tetrapods grouping together, the temnospondyls being placed as a group and branching from the stem-amniotes. The similarity of our retrieved branching pattern to previous analyses shows that the early tetrapod phylogeny may potentially be becoming more stable in terms of order (temnospondyl, colosteid, etc.) (Ruta & Bolt, 2008; Ruta & Coates, 2007; Pardo et al., 2017). Also, the new early tetrapod discoveries having so much impact on the tree shows that the make-up of these orders still have the potential to change drastically. The results from this project, as well as the database of characters compiled can be used as a stepping stone in order to increase understanding into the early tetrapod group as a whole. The introduction of post-cranial characters would be the next step in order to do this, as well as including an expanded taxa list.

3. The Evolution of Morphological Disparity in the Radiation of Early Tetrapods

Abstract

The modern diversity of phenotypic plans, seen in nature globally, are the result of active processes of evolution of disparity accumulated during the proliferation of animal lineages. Morphological disparity is concerned with differences in morphology between groups of individuals or taxa. There have been surprisingly few studies of morphological disparity for early tetrapods, even due to the group undergoing many evolutionary events such as the colonisation of a new ecospace. This study aims to rectify this lack of studies by aiming to quantify the disparity among Palaeozoic early tetrapod skulls, using discrete characters, with the expectation that a pattern similar to that of the phylogeny will be observed. We also aim to evaluate the overall pattern of species distribution in morphospace; is there more significant clustering or dispersal among species than we would expect at random? Finally, we aim to assess the amount of disparity through time hypothesising that disparity will peak at the beginning of the lineage. Our study finds that early tetrapod disparity does follow a pattern similar to that of the phylogeny. Early tetrapods are clustered in morphospace, while the three groups (stem-tetrapods, stem-amphibians and stem-amniotes) are statistically separate from one another. This shows that within groups early tetrapods are morphologically similar but between groups they are morphologically different. The stem-amphibians and stem-tetrapods also occupy separate corners of the morphospace, away from the stem-tetrapods, showing that at the start of a lineage there is an opportunity to explore new areas of morphospace. Disparity through time contradicts our hypothesis and shows a decreased amount of disparity at the start of the lineage. Further study with an increased taxon set would be the next step in better understanding morphological disparity of the early tetrapod group.

3.1 Introduction

The macroevolutionary history of a clade is the result of a complex interplay between models of speciation and extinction as well as patterns of morphological variation. Disparity (the range of phenotypic variety) permits exploration of deep time models of evolutionary transformation and is therefore key to our understanding of a variety of biological processes, both in the distant past and at present. Opposed to diversity, which takes into account number of species, genera or higher taxa, morphological disparity is concerned with differences in morphology between groups of individuals or taxa (Harper, 2000; Oyston *et al.,* 2015). A consensus on a definition for disparity has still not been agreed upon, nor an agreed way in which to measure it. There are two distinct categories in which analyses of disparity are carried out; methods that either have a phylogenetic context, which rely on the data that have been used to assemble phylogenies (cladistic) or methods that are detached from any phylogenetic hypotheses, that rely on measurements as well as discrete characters that may or may not have phylogenetic utility (phenetic) (Wills, 2001). Disparity can be quantified by using a number of different input data: discrete characters (Foote, 1994; Young *et al.,* 2010), geometric morphometric landmarks (Brusatte *et al.,* 2012; Davis *et al.,* 2016; Werneburg *et al.,* 2014) and measurements (Dyke *et al.,* 2009).

There are patterns of change over geological time that disparity can take from the origin of a lineage to extinction (Colombo *et al.,* 2015; McShea, 1994). However, previous disparity studies have shown that the most common trajectory is for clades to reach their maximum disparity early in their histories (Foote, 1996; Hughes *et al.,* 2013; Oyston *et al.,* 2015). This is thought to be due to ecological or developmental reasons; there is an increased chance to diversify more rapidly at the start of a radiation due to constraints upon the morphospace being lifted. This could be due to other occupants leaving the ecosystem due to an extinction event or taxa being able to access a resource that was previously inaccessible, due to acquiring a new adaptation (Hughes *et al.,* 2013). Early tetrapods are an example of a group acquiring a new

adaptation that may have helped them access a resource that was previously unobtainable. The acquisition of terrestrial locomotion as well as adaptations for breathing would potentially allow the group to explore a new wider area of morphospace. This has previously been shown to be the case in early tetrapods, with a release of characters due to the invasion of new habitats (Wagner *et al.,* 2006). However, even due to this there is still a scarcity of analyses of disparity in early tetrapods.

Palaeozoic early tetrapod skulls are diverse in their morphology, with many 'unusual' forms (Schoch & Milner, 2014). For example, *Greererpeton* is a fairly basal Carboniferous species and a member of the tetrapod group known as Colosteidae, usually placed in a derived position on the tetrapod stem group. It has a very flattened skull, and is primitive in many respects, e.g. in the dorsally orientated elliptical orbits, the presence of gill bars, the condition of the vertebrae with a dominant anterior ventral element (intercentrum) shaped like a wedge and two posterior dorsal triangular elements (pleurocentra) (Smithson, 1982). *Balanerpeton woodi*, a species of early temnospondyl tetrapod from the Carboniferous, has a rounded skull with large orbits (Milner & Sequeira, 1993). On the other hand, the cochleosaurid temnospondyls, such as *Cochleosaurus bohemicus* and *Cochleosaurus florensis,* also from the Carboniferous, have long slender skulls reminiscent of a crocodile (Rieppel, 1980; Sequeira, 2003). The baphetids, including *Megalocephalus*, have peculiar 'key-hole' shaped orbits that are very different to the rounded orbits of the rest of the early tetrapod taxa (Beaumont, 1977). Early tetrapod skulls also change significantly during the Palaeozoic with basal aquatic Devonian taxa having a very different skull morphology than taxa later on (Clack & Milner, 2015; Coates *et al.,* 2008; Schoch & Milner, 2014). For these reasons, a study of cranial disparity that takes into account the rich and ever-growing databases of skeletal characters will assist in quantifying the subtle variations that exist across and within groups.

Disparity has been used in many studies to visualise morphological similarities or differences between a wide range of fossil taxa (Brusatte *et al*., 2008; Foth & Joyce, 2016; Halliday &

Goswami, 2016; Wills *et al.,* 2012). However, early tetrapods are under-represented in these investigations of disparity. This is surprising as they are a diverse group of animals, that went through many morphological changes accompanying major ecological transitions; and therefore, it is nearly unquestionable to interrogate the character database of these organisms to determine patterns of phenotypic change (Coates *et al.,* 2008). One of the few studies carried out on the disparity of early tetrapods used the appendicular skeleton to compare levels of disparity to that of fish (Ruta & Wills, 2016). It was found that tetrapods attained similar levels of disparity to fish even due to large the large diversity of early tetrapod appendicular morphology.

Many studies of disparity using cranial characters has been undertaken for other fossil groups but not for early tetrapods (Butler *et al.,* 2012; Pierce *et al.,* 2008; Wroe *et al.,* 2000). This chapter aims to rectify this lack of studies by investigating disparity in Palaeozoic early tetrapods using the most comprehensive database of cranial characters to date. One point of inquiry is whether early tetrapods in the Palaeozoic follow a pattern of high disparity at the start of their clade. This will be investigated by plotting disparity through time using taxa from the start of the early tetrapod lineage in the Devonian as well as taxa further removed from it later in the Palaeozoic. Also, how taxa are placed within morphospace will be investigated using principal coordinate analysis and spatial point analyses.

This chapter aims to:

i. Quantify the disparity among Palaeozoic early tetrapod skulls using discrete characters. We hypothesise that a pattern similar to that of the phylogeny will be observed, with species closer together on the phylogeny clustering closer together in morphospace. However, we also expect to possibly see a more nuanced pattern that reflects not just phylogenetic branching but also the amount of noise (homoplasy) in the data, as well as differences between species that belong in the same group.

ii. Evaluate the overall pattern of species distribution in morphospace - Is there more significant clustering or dispersal among species than we would expect at random? While the taxon sample may be limited, it will nonetheless provide an initial characterization of areas of overlap and/or gaps between groups. These, in turn, could pave the way to future explorations of patterns of character divergence.

iii. Assess the amount of disparity through time. We hypothesise that disparity will peak at the beginning of the lineage. With this, we aim to evaluate modalities of character partition among subclades over time. Are there periods during which tetrapod subclades tend to invade each other's regions of morphospace, and others when subclades tend to be confined to smaller and non-overlapping regions?

3.2 Method

3.2.1 *Principal Coordinate Analysis*

Principal Coordinates Analysis (PCoA) is a multivariate analysis that can be used to visualize similarities and differences between taxa. PCoA starts with a tabulation of inter-taxon distances, and seeks to represent the original distances among species in a lower dimensionality mathematical space delimited by PCo axes (Wills, 2001; Young *et al.,* 2010). A distance matrix of dissimilarities (in character states) between taxa was produced using the character database (Appendix 3) (Gower, 2014). PCoA was then applied using those distances.

The PCoA was carried out in the R language (R core team, 2012), using the Claddis library (Lloyd, 2016) to produce a set of Generalized Euclidean distances among taxa by correcting for missing entries, and then ape library (Paradis *et al.,* 2004) to perform the PCoA and extract coordinates (PCo scores), which were utilized in disparity analyses and to plot a morphospace. In Claddis the MorphoDistMatrix command was used in order to calculate a matrix of

generalized Euclidean distances (GED). In ape, the Cailliez method was enforced to correct negative eigenvalues (Cailliez, 1983). The first three coordinate axes from the PCoA were used to build three morphospace plots in the program PAST v3.14 (Hammer *et al.,* 2001).

3.2.2 *Clustering and Dispersal*

To visualise differences in the density of taxa in the character morphospace on the first two axes of the PCoA, spatial point analyses were carried out. Point pattern analyses are a class of statistics designed to analyse patterns of objects distributed in any numbers of dimensions (Illian *et al.,* 2008). The first, Ripley's K, is a non-parametric function used to evaluate the departure of a set of points from a random distribution. If points are distributed at random, e.g. in a 2D plane or a 3D space, then an approximately identical number of points will be found in a circle (respectively, a sphere) of a given size placed in different positions in the morphospace. The number of points will change with the size of the circle or the sphere, but will be approximately constant for a circle (or sphere) of a fixed size and in different positions in the morphospace (Dixon, 2002). In this study, Ripley's K is being used to test whether the observed points (plotted taxa) are consistent with a homogeneous Poisson Process (complete spatial randomness) or whether they appear over-clustered or over-dispersed. Ripley's K was carried out via PAST v3.14 using the first two axes from the PCoA and plotted in the form of a graph. The graph shows the Ripley's K function for the real data and a set of K functions that depict random data point additions. These K functions are used to generate a confidence intervals (1000 replicates were used) and a median K function. The K function built from the real data is then compared to the randomly generated K functions. If the data points are above the upper boundary of the distribution (null- Poisson process), then the points are significantly more clustered than we would expect at random. If the data points are below the lower boundary (null- Poisson process), then the points are significantly dispersed. The observed K function may intersect the randomly distributed ones several times, e.g. showing overclustering or overdispersal at different scales of spatial inter-taxon distances as well as a non-significant

deviation from randomness. Significant clustering indicates more similarity between taxa than expected and significant dispersal indicates taxa are less similar than expected.

Nearest neighbour's analysis is another way in which to analyse spatial point process data and assess if points are significantly more dispersed or clustered. However, it simply provides a way to evaluate overclustering or overdipersal within the window of observations, without any information on the pattern of variation at small or large spatial distances (Hammer *et al.,* 2001). A nearest neighbour distance simply takes into account the shortest Euclidean distance between two points, then the next shortest distance between either or none of the first two points. Nearest neighbour distances were calculated via the program PAST v3.14. The analysis was carried out using the smallest rectangle area estimation which simply looks at the smallest rectangle all of the points will fit inside of (Hammer *et al.,* 2001). Significance is based against whether the observed nearest neighbour distances are different to the null hypothesis of a random pattern of clustering (Poisson process). Finally, a one-way PERMANOVA was carried out, using the first two PCo axes, in order to quantify whether there is significant clustering between groups. A PERMANOVA (permutational analysis of variance) compares the distances between taxa groups with a null in which all distances are the same (Anderson, 2001). The number of permutations used for the calculation was 9999. A pair-wise PERMANOVA was conducted using the first two PCo axes, to assess whether the groups of taxa (the stem-tetrapods, the stem-amphibians and the stem-amniotes) are significantly separated from each other.

3.2.3 *Disparity through time*

Disparity through time (DTT) was modelled for our data using our time calibrated tree (fig. 8), our character dataset (Appendix 3) and was analysed using the geiger library (Harmon *et al.,* 2008) in R. DTT calculates the mean disparity of morphology on a tree then compares it to the expected amount of disparity under a Brownian motion model. Brownian motion is sometimes known as random evolution or a neutral- drift model and is used in this context as a null for the

amount of disparity at random (Pagel, 1999). If the observed amount of disparity is higher than the expected value under Brownian motion, the taxa occupy a larger proportion of trait space than random. If the observed amount is below the expected value, taxa occupy isolated areas of morphospace. If the observed disparity is outside the 95% confidence interval then the amount of disparity can be said to be significantly different from random.

The morphological disparity index (MDI) was calculated from the data. This quantifies the overall difference in relative disparity for a trait compared with the expected under a Brownian motion model (Slater *et al.,* 2010). Negative MDI indicates lower than expected disparity compared to Brownian motion, whereas positive MDI indicates higher disparity (Harmon et al., 2003). Specifically, MDI is the difference between the DTT curve for the observed data and the median curve generated from several random simulations of Brownian motion disparity. If the observed DTT curve is higher than the median DTT, then subclades tend to occupy large and overlapping regions of trait space. In the opposite scenario, with observed DTT below the median DTT, subclade morphology is strongly partitioned, such that subclades tend to occupy small and non-overlapping regions of morphospace.

3.3 Results

3.3.1 *Principal Coordinate Analysis*

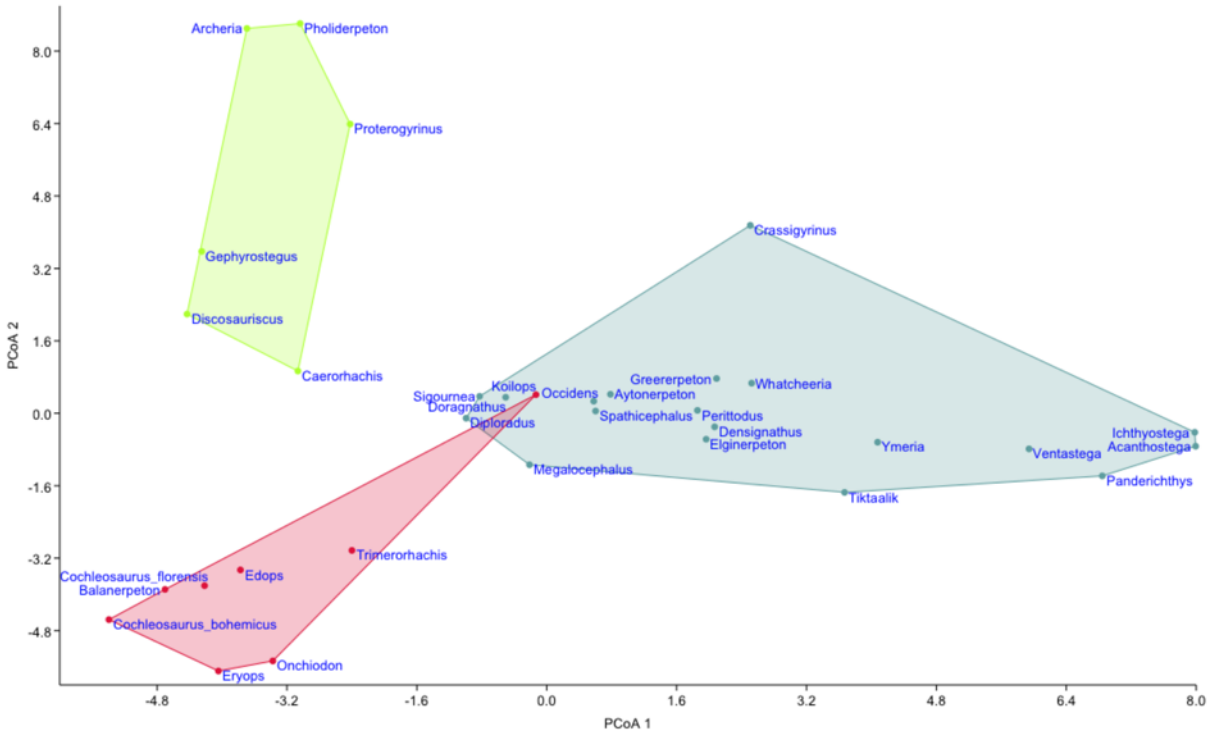
Morphospace plots for the first three coordinates from the PCoA are shown in fig. 10. The first axis explains 8.6% of the variation in the data, while the second axis accounts for 6.8% (these values are low due to the correction for negative eigenvalues decreasing the variance of the first few axes). The plot of the first and second axes, shown in fig. 10a, shows a clear separation between the stem-amphibians (red) and the stem-amniotes (green). The stem-tetrapods (blue), are mainly concentrated in the centre of the morphospace with an obvious outlier represented by *Crassigyrinus*. Four of the more basal early tetrapods, *Ichthyostega, Acanthostega,*

Ventastega and Panderichthys form a group of their own away from the majority of the other taxa. The plot does reflect the retrieved phylogeny (fig. 6) closely. The biggest difference is in respect to the placement of *Koilops*. In the phylogeny, *Koilops* is placed within the temnospondyls in a clade with *Trimerohachis*. The PCoA plot shows *Koilops* plotted away from the temnospondyl group, in amongst the stem-tetrapods. This placement is causing an overlap between the stem-amphibians and the stem-tetrapods.

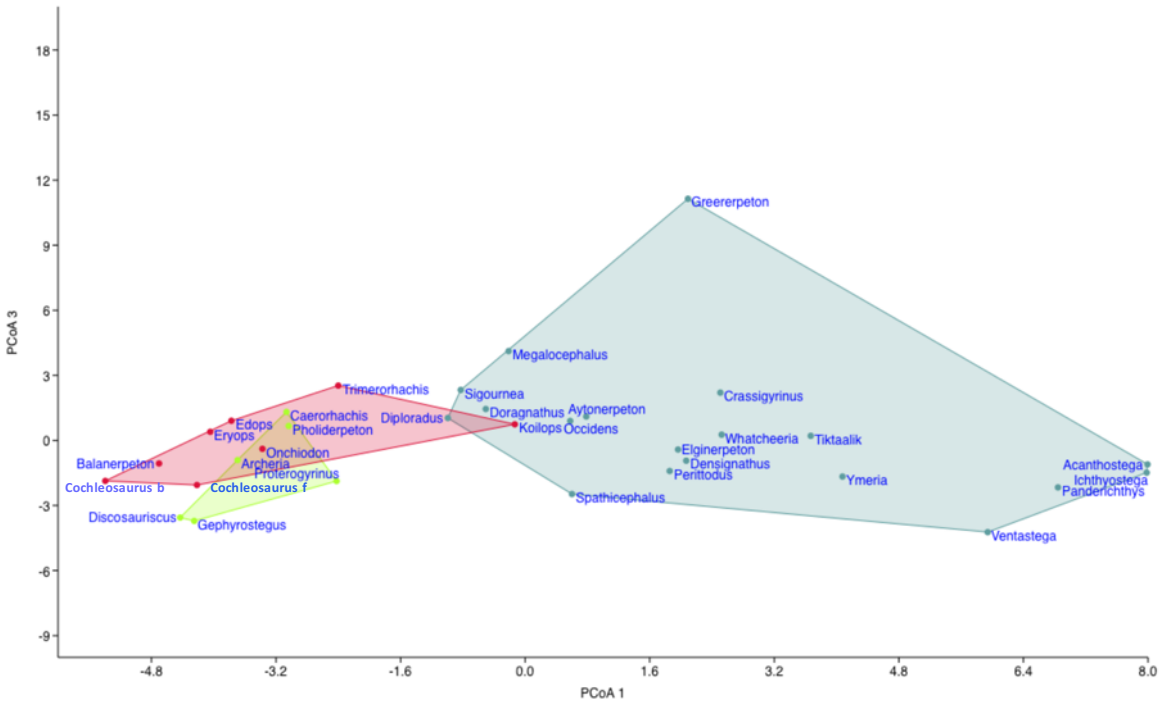
The morphospace plot of the first versus third axis (fig. 10b) shows an overlap between the stem-amphibians and stem-amniotes. The third axis explains 4.5% of the variation in the data. The stem-tetrapods are placed on the right-hand side of the plot, similarly to the placement in the plot for the first and second axes (fig. 10a). Also, the stem-amphibians have an overlap with the stem-tetrapods, again due to the placement of *Koilops* with the stem-amphibians. Finally, the stem-amphibians and the stem-tetrapods cover the largest area out of the three groups.

The morphospace plot for the second versus third axis (fig. 10c) shows quite a different picture, with the stem-tetrapods placed in the middle of the stem-amniotes and the stem-amphibians rather than to the right-hand side as seen on the previous plots. Also, the stem-tetrapods are spread over a much smaller area than in the previous plots. In this PCoA plot the stem-amniotes have an overlap with the stem-tetrapods due to *Caerorhachis*.

a)


Archeria
Pholiderpeton
Proterogyrinus
Gephyrostegus
Discosauriscus
Caerorhachis
Crassigyrinus
Greererpeton
Whatcheeria
Sigournea
Koilops
Occidens
Aytonerpeton
Doragnathus
Spathicephalus
Perittodus
Diploradus
Densignathus
Elginerpeton
Ymeria
Ichthyostega
Acanthostega
Ventastega
Megalocephalus
Panderichthys
Tiktaalik
Trimerorhachis
Cochleosaurus_florensis
Edops
Balanerpeton
Cochleosaurus_bohemicus
Onchiodon
Eryops
PCoA 2
PCoA 1
8.0
6.4
4.8
3.2
1.6
0.0
-1.6
-3.2
-4.8

b)


Greererpeton
Megalocephalus
Trimerorhachis
Sigournea
Crassigyrinus
Caerorhachis
Diploradus
Doragnathus
Aytonerpeton
Edops
Pholiderpeton
Koilops
Occidens
Eryops
Whatcheeria
Tiktaalik
Onchiodon
Elginerpeton
Balanerpeton
Archeria
Densignathus
Acanthostega
Proterogyrinus
Perittodus
Ichthyostega
Cochleosaurus b
Cochleosaurus f
Ymeria
Panderichthys
Spathicephalus
Discosauriscus
Gephyrostegus
Ventastega
PCoA 3
PCoA 1
18
15
12
9
6
3
0
-3
-6
-9
-4.8
-3.2
-1.6
0.0
1.6
3.2
4.8
6.4
8.0

c)

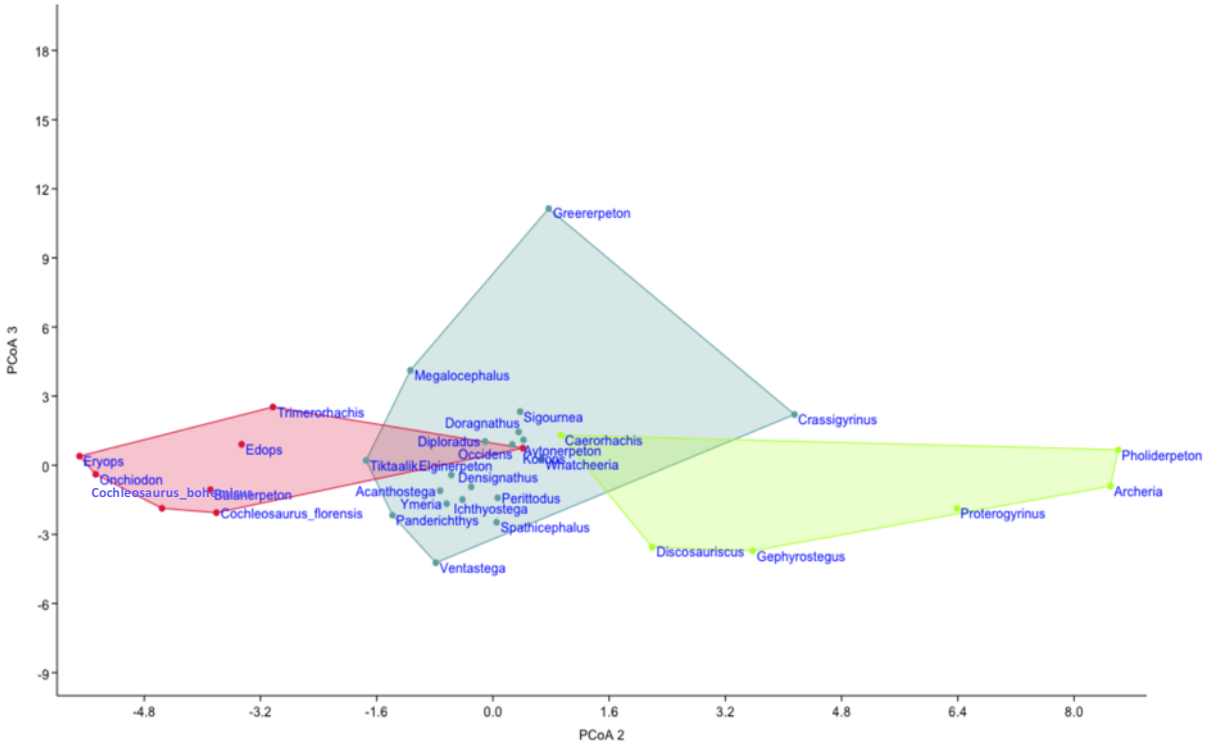


Fig. 10. Principal coordinate analyses (PCoA) using the first three PCoA axes: (a) axis 1 & axis 2; (b) axis 1 & axis 3; (c) axis 2 & axis 3. The three groups are shown by the colours stem-tetrapods (blue), stem-amniotes (green) and stem-amphibians (red).

3.3.2 *Clustering and Dispersal*

The Ripley's K analysis is shown in fig. 11. This plot can be interpreted with the y-axis showing the k values at each distance, while the x-axis shows the different spatial distances the points are analysed within. Analysing the points within multiple spatial distances allows the potential for more patterns to be unearthed, than when compared to a process using just one distance. Our plot shows that the observed k function (amount of clustering) is above the top predicted k function (amount of clustering) for the majority of the plot. This indicates that there is significant clustering among taxa. The nearest neighbour's analysis also retrieves statistically significant clustering ($p = 0.0002$) using the smallest rectangle area estimation (fig. 12). The axes in this plot shows the number of points that have nearest neighbours of a certain distance measurement on the morphospace plot. This is different from the Ripley's K plot due to nearest

neighbour analyses being investigated using the whole plot rather than using different size distance 'frames' that Ripley's K uses. The analysis shows that the majority of points have nearest neighbours of the smallest distance, indicating clustering of points. Both of these analyses indicate that Palaeozoic early tetrapods are more clustered in morphospace than expected at random.

The PERMANOVA indicates that there is an overall significant amount of clustering ($F = 30.6$, $p < 0.0005$). Also, the three groups (stem-tetrapods, stem-amphibians and stem-amniotes) are all significantly separated from each other according to the pair-wise PERMANOVA (table 2).

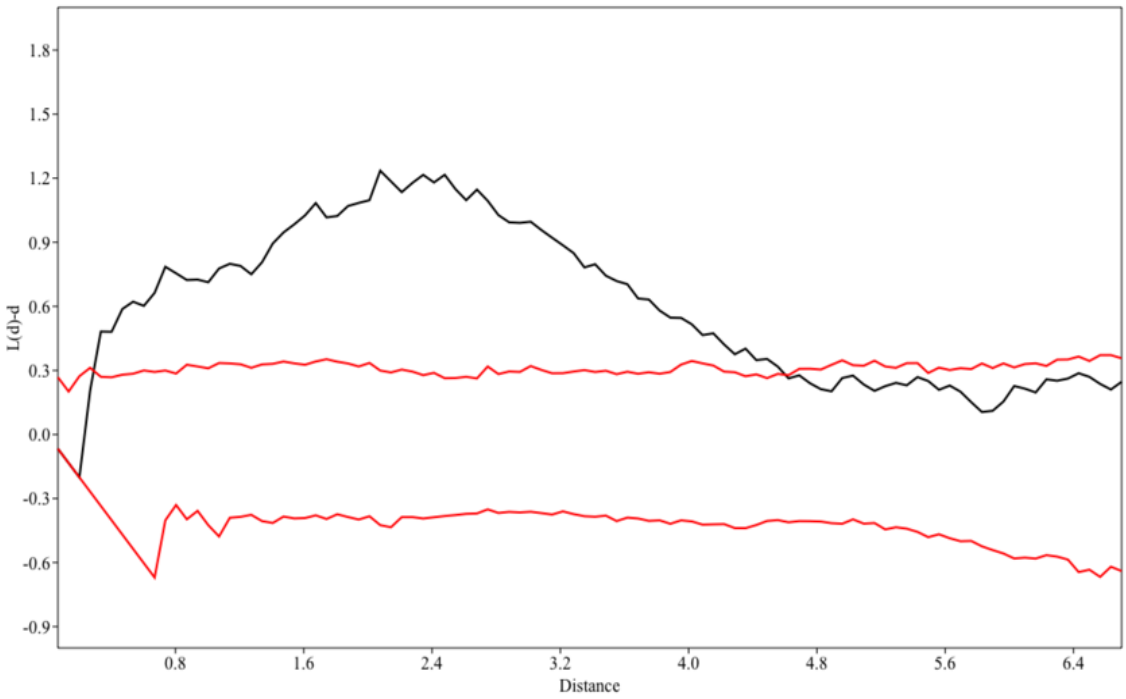


Fig. 11. Ripley's K plot showing amount of clustering or dispersal between taxa. The black line indicates the observed amount of clustering or dispersal. The two red lines indicate the upper and lower boundaries.

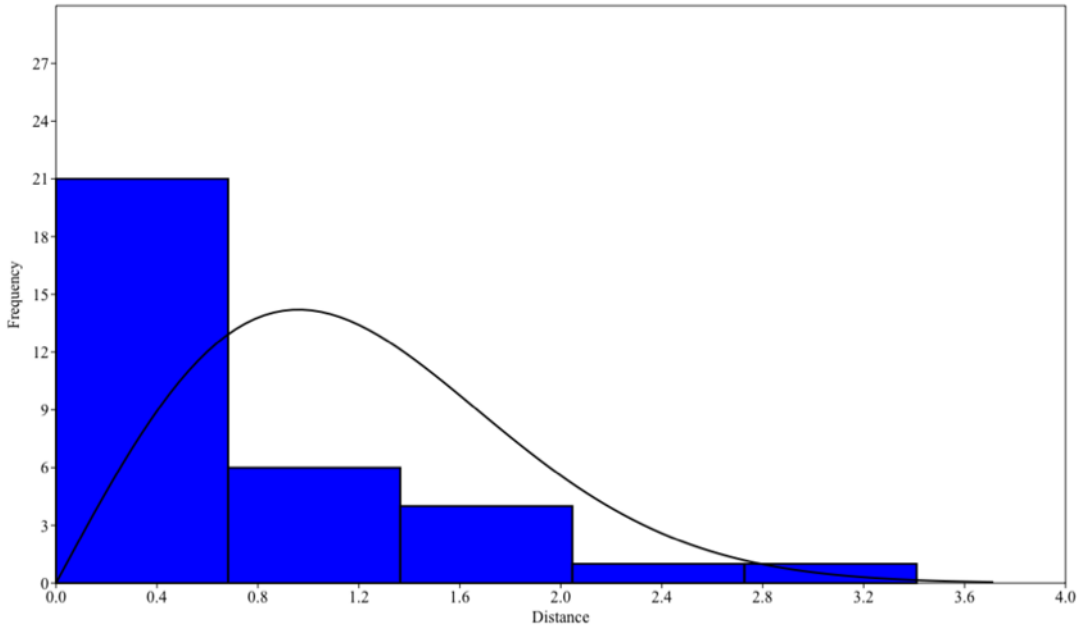


Fig. 12. Nearest Neighbour Analysis using the smallest rectangle area estimation and wrap-around edge correction. The black line shows the expected nearest neighbour distances. The blue bars show the observed nearest neighbour distances.

	Stem-tetrapods	**Stem-amniotes**	**Stem-amphibians**
Stem-tetrapods	X	0.0003	0.0003
Stem-amniotes	0.0003	X	0.0009
Stem-amphibians	0.0003	0.0009	X

Table 2. Pairwise PERMANOVA showing bonferoni – corrected p values for the amount of separation between each of the three groups (stem-tetrapods, stem-amphibians and stem-amniotes).

3.3.3 *Disparity through time*

The disparity through time plot is shown in fig. 13. We have used a relative time scale in this plot, a scale which has been used by many previous studies (Frédérich *et al.,* 2012; Koecke *et al.,* 2013; Slater *et al.,* 2010). The scale can be interpreted as millions of years ago from the first appearance datum (FAD) of the oldest taxa at 0 (in this case *Panderichthys rhombolepis*

with a FAD of 383.7 mya) to the FAD of the youngest taxa at 25 (in this case *Trimerorhachis insignis* with an FAD of 290.1 mya), the middle increments are equal proportions between these two dates. The MDI is 0.043 indicating that, overall, subclades tend to occupy larger areas of trait space than expected. However, this difference is not statistically significant, as the observed DTT occurs for the most part inside the 95% confidence envelope built from 1000 random simulations. One exception to this, however, is seen right at the start of the plot, where the observed DTT is lower than expected under Brownian motion and the 95% confidence interval, suggesting early strong partitioning of trait space. There is also a small period of time where the observed disparity is higher than the amount of disparity expected under a Brownian

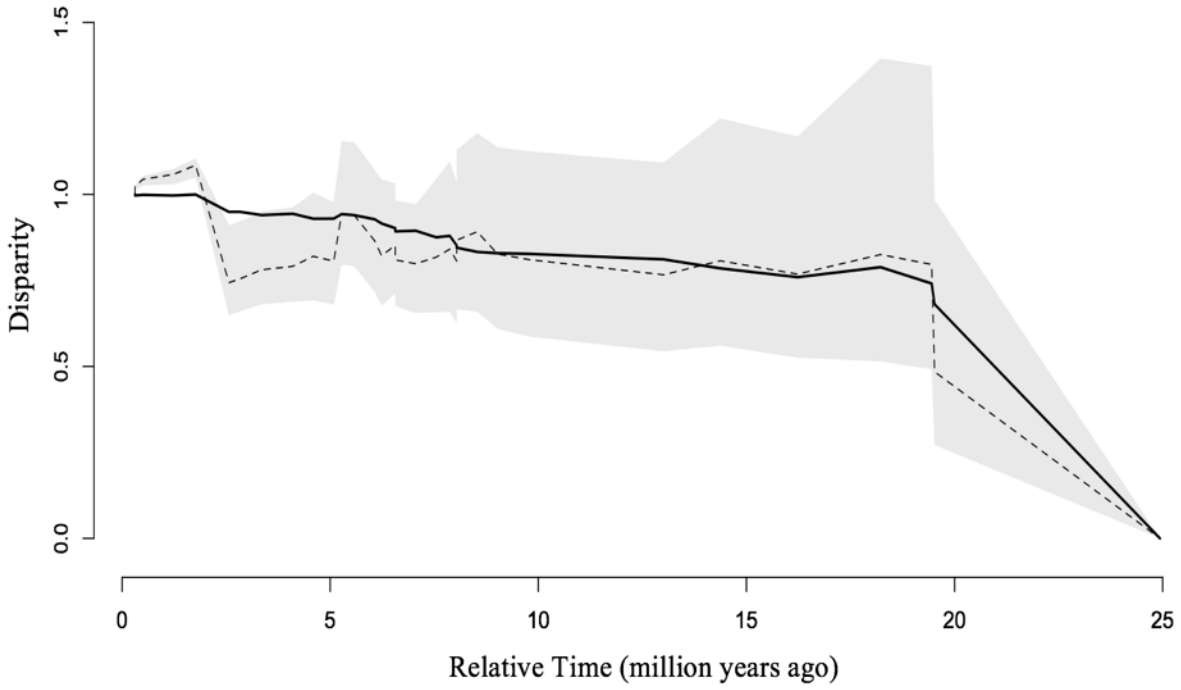


Fig. 13. Disparity through time plot (MDI= 0.043). The solid black line shows the observed amount of disparity. The dotted black line shows the expected amount of disparity under a Brownian Motion model and the grey area shows the 95% significance interval.

3.4 Discussion

This study provides an analysis of disparity for early tetrapod cranial traits, an area that is greatly lacking for the group. By using a comprehensive dataset, the results of this study show that early tetrapods show a pattern of disparity similar to the phylogeny, with the three groups (stem-tetrapods, stem-amphibians and stem-amniotes) being significantly separated from each other in morphospace. This study also shows that within these groups the species are significantly clustered showing different morphologies between groups and more similar morphologies within groups.

The first character morphospace (fig. 10a) matches up with the phylogenetic tree (fig. 6) closely, showing the distinct splitting event between the stem-amphibians and stem-amniotes. However, a notable exception is the placement of *Koilops* within the stem-tetrapods, rather than the temnospondyls as it is on the phylogeny. This pattern is seen throughout all three morphospace plots. This could be due to the small amount of characters it could be coded for as it is only known from one incomplete skull specimen alone (Clack *et al.,* 2016). This indicates further that the placement of *Koilops* amongst Temnospondyls may not be the best position for the species and a placement with the stem-tetrapods may be more suitable. Further discoveries of *Koilops* specimens would vastly increase our knowledge of the species and could potentially cement its position on the early tetrapod tree. Four of the most basal of the taxa (*Panderichthys, Ventastega, Acanthostega, Ichthyostega*) are grouped away from the rest of the stem tetrapods in the first two plots (fig. 10 a & b). *Tiktaalik,* another basal tetrapod, is placed outside of this grouping which is interesting as it has both fish and tetrapod characteristics (Clack & Milner, 2015). Due to this it would have been predicted to have grouped close to *Panderichthys*, a sarcopterygian fish, as it is on the phylogeny. These exceptions to the morphospace reflecting the phylogeny show that the two may not always match. The three morphospace plots show varying patterns of disparity. However, the stem-tetrapods do cover

the largest area out of all three groups over all the plots. This is perhaps due to larger sample of stem-tetrapods used in this study. However, this could also indicate that the stem-tetrapods show a larger range of morphologies compared to the more specialised stem-amphibians and stem-amniotes.

The disparity through time plot shows a pattern of disparity in which subclades tend to occupy larger areas of trait space than expected, however, this difference is not statistically significant. At the start of the lineage disparity is lower than expected under Brownian motion. This contradicts the hypothesis of a high level of disparity at the start of a lineage (Hughes, *et al.,* 2013). The low levels of disparity at the start of the group is potentially due to the taxa during this section of time being morphologically similar. Many of the most basal taxa such as *Ventastega, Acanthostega* and *Ichthyostega* share many characteristics due to a similar habitat and diet which could explain the significant low disparity at the beginning of the lineage (Ahlberg & Clack, 1998).

Early tetrapod craniums show a significant amount of clustering on the morphological plot, signifying that they are more similar than dissimilar. A lower level of disparity than expected could be due to the taxa chosen potentially being of a similar morphology. However, the taxa chosen cover most of a large amount of the early tetrapod diversity in the Palaeozoic, with at least one member from each group covering the transition. Another explanation could be that perhaps the characters chosen may not have covered the disparity well or perhaps some areas of the cranium are still underrepresented in the compiled dataset. There is a significant amount of separation between the three groups (stem-tetrapods, stem-amphibians and stem-amniotes). This indicates that within clades early tetrapod taxa are morphologically similar with similar cranial compositions, however, across clades they are more morphologically different (Coates & Clack, 2012).

The results from this study indicate a lower level of disparity than expected for a clade at the beginning of its lifetime with taxa within clades being more clustered than dispersed. Ruta & Coates (2016) gave a possible explanation for low disparity in the appendicular skeleton of early tetrapods being that the journey from water to land could have placed constraints upon the range of limb diversity. Perhaps this is a similar reason for the low disparity in cranial characters; the novel function of breathing oxygen as well as a change in diet could have put constraints upon the range of cranial diversity available, however this may not necessarily be the case (Pérez-Barbería & Gordon, 1999). Also, a smaller sample taxa further removed from the transition could have magnified this effect for the overall disparity.

In conclusion, Palaeozoic early tetrapod craniums show a significant amount of clustering between taxa within clades, but there is separation between the three groups investigated in this study. At the start of the lineage there is a significant reduced amount of disparity compared to random, contradicting previous hypotheses of high disparity at the start of lineages (Hughes *et al.,* 2013; Oyston *et al.,* 2015). Using an increased taxon list as well as an expanded dataset to include post cranium characters would be the next step in further delving into disparity in relation to the Palaeozoic early tetrapod group.

4. Tempo and mode of macroevolutionary diversification of traits in early tetrapods

Abstract

Remarkable advances in computational evolutionary studies has caused an explosion in investigations of rates of trait evolution. However, some groups are still lacking in this kind of macroevolutionary investigation, for example, the early tetrapod group. This study aims to fill this gap by assessing the rate of character trait change in the skull across the early tetrapod clade, looking at both changes on specific branches and on the tree as a whole. Principal coordinate analysis and the motmot and Auteur R packages were employed in order to carry out these aims. Across the tree instances of increased rate were found that coincided with important speciation events. The beginning of the lineage shows an increased rate of evolution in both methodologies. This is also the case at the splitting event between the stem-amniotes and stem-amphibians. An early burst model of evolution was retrieved on the first PCo axis, backing up the presence of high rate at the start of the lineage further. Overall early tetrapod cranial data shows a variety of patterns of the rate of character state transformation over the lineage, however, there are instances of corroboration between analyses. The most important being that both of the periods in which tetrapods invade a new ecospace, at the fish-tetrapod transition and at the amphibian-amniote split, show an increased rate of evolution backing up the idea of character release at the start of lineages.

4.1 Introduction

Ever since Darwin evolutionary biologists have endeavoured to measure the pace of evolution, i.e. the chronology and patterns of evolutionary transformation. In recent years, with

remarkable advances in computational evolutionary studies and the production of sophisticated software, rates of trait evolution have become part of mainstream macroevolutionary investigation. Understanding the rate of evolution (the accruing of change per unit time) ultimately informs our knowledge of how biodiversity was shaped over millions of years (Adams, 2012). The overall pattern of evolutionary change can be explained by a certain general model of evolution (e.g. Brownian motion, Lambda, Ornstein-Uhlenbeck, etc.) and by more specific analyses of rate through sections of a tree. General models, while valuable, only provide an approximate estimate of the evolutionary pace, but disregard nuances of branch or time-specific patterns.

It has been shown by mathematical models that rates of morphological evolution are inversely correlated with niche spaces available i.e. an adaptive radiation (the evolution of species as a consequence of adaptation to new ecological niches) (Colombo *et al.,* 2015; Gavrilets & Losos, 2009). This leads to an 'early burst' model of evolution being predicted as the most prevalent, showing a high rate of morphological change at the beginning of a clade due to the availability of vacant niches to fill, with a slowing of rate being observed later on in the clades history (Colombo *et al.,* 2015). However, an early burst model has been found to not always be the best supported model of an adaptive radiation (Harmon *et al.,* 2010).

Studies of rate have been applied to a wide variety of organisms (Benson & Choiniere, 2013; Fischer *et al.,* 2016; Mahler *et al.,* 2010; Mooers *et al.,* 1999; Vidal-García & Keogh, 2017). These studies have uncovered varying patterns of rate of evolution and have been investigated with a variety of macroevolutionary traits (Arbour & López-Fernández, 2013; Brusatte *et al.,* 2008(2); Caumul & Polly, 2005; Harmon *et al.,* 2010). However, similar studies in early tetrapods remain in their infancy even due to the group undergoing many important evolutionary transitions. One of the few studies investigated the rate of character state change using the whole skeleton (Ruta *et al,* 2006). A decrease in rate over time was found both when measured per cladistic branch and per million years along phylogeny. This was the first time

this pattern was retrieved for terrestrial vertebrate taxa and was thought to have been potentially due to low constraints, early on in the clades history. Another study used the mandibles of Palaeozoic early tetrapods in order to investigate rates of biomechanical evolution (Anderson *et al.,* 2013). The study found that there is a later diversification of mandibular function in tetrapods. An investigation of morphological rate using the whole suite of characters for the cranium has never been undertaken before. We aim to rectify this by exploring rates of evolution for Palaeozoic early tetrapods using the most comprehensive database of cranial characters to date.

The resolution of early tetrapod interrelationships has improved over the past few decades despite the lack of overall consensus on specific domains of tetrapod diversity (Ahlberg & Clack, 1998; Clack *et al.,* 2016; Clack, 2002; Ruta & Coates, 2007). This enhanced knowledge invites analyses that delve further into the groups relationships, including studies of evolutionary rates which are still scarce for the group. The early tetrapods are favourable to analyses of rates for many reasons. Firstly, Palaeozoic early tetrapods underwent some of the most dramatic transformations ever documented at the onset of major adaptive radiations, given the radical structural, functional, and ecological shifts that underpinned the colonisation of land (Coates *et al.,* 2008). Secondly, early tetrapods also undergo episodes of major diversification in the splitting event between stem-amphibians and stem-amniotes. This saw the stem-amphibians continue to occupy an aquatic habitat while the stem-amniotes mark the first invasion of fully terrestrial habitats (Ruta *et al.,* 2006).

The aims of this chapter are:

i. Assess the rate of character trait change across the tree by using two methodologies. We hypothesise that significantly high rate of character state transformations occurred

close to the origin of limbed vertebrates of the clade and at the separation between stem amphibians and stem amniotes.

ii. Find the best fitting model of evolution to the tree to infer the general pattern of rate change. This allows us to identify the overall evolutionary rate which is useful in showing estimates of evolutionary pace along the whole tree.

4.2 Method

4.2.1 *Rates of Evolution*

The rate of character state evolution is commonly analysed in studies of evolutionary rate. This takes into account how many character state transformations occur per million years, i.e. how many times a character (see Appendix 2) changes from one state to another. Two different R packages were used to analyse rates of character evolution on our chosen tree (fig. 6). The outputs from each of the analyses were compared in order to give a more detailed picture of rate change along branches. In both cases, a time-calibrated version of the tree was required (fig. 8).

a) Analysis of Rates in Motmot

Using the time calibrated tree (fig. 8.) and the first three axes from the previously calculated PCo scores (chapter 3.2.1), rates of trait evolution were quantified using the transformPhylo.ML function (models of trait macroevolution on trees) in Motmot (Thomas & Freckleton, 2012). From this the ML Rate is reported which measures the amount of trait change on branches compared to a background rate. This was used in order to assess the amount of rate decrease or increase amongst clades. An ML Rate above 1 indicates a speeding up of the rate of trait change and an ML Rate below 1 indicates a slowing down of rate. A colour-coded representation of shift magnitude on the tree was produced in the same package, showing the separate rate shifts (if any) on the tree.

b) Analysis of Rates in Auteur

Rate of character state transformation were assessed using the Auteur (Accommodating Uncertainty in Trait Evolution Using R) package in R (Eastman *et al.,* 2011). Auteur uses a Bayesian framework to detect if there are any shifts on a tree, either on individual terminal branches, across the entire phylogeny, or at an internal branch. A Bayesian framework takes into account the observed data as well as the likelihood (Huelsenbeck & Ronquist, 2001). Using the time calibrated tree (fig. 8) and the first three PCo axes (chapter 3.2.1) an analysis of rate was carried out. A reversible - jump Markov Chain Monte Carlo Analysis (Green, 1995) with two chains is carried out with one million iterations carried out for each of the three axes. Outputted is a tree with coloured branches indicating rate increases and decreases. Grey branches indicate that the branch has a rate equal to the background rate of evolution. Branches in red have a rate higher than the background rate, the deeper the red the higher the rate compared to the background. Branches shown in blue have a rate slower than the background rate, the deeper the blue the lower the rate than the background rate. The size of the circles on nodes represent the probability of the shift to have occurred. The larger the circle the higher the probability of a shift to have occurred. The colour of the circle indicates that at that node there was a change of rate from either high to low or low to high. Thus, a darker red the circle indicates there is a higher upturn in rate, whereas a darker blue the circle indicates a downturn in rate. Alongside colours, branches are outputted with values that indicate direction of the shift on a scale from -1 to 1. Minus values indicate a decrease in rate and positive values indicate an increase in rate for that branch.

4.2.2 *Models of Evolution*

The statistical environment R (3.2.0) was used to identify which model of evolution provides the best explanation for the PCo score distribution across taxa (chapter 3.2.1). The analysis was carried out using the fitContinuous function in Geiger and the first three PCo axes. The fit of nine statistical models were compared to assess which model of evolution best explains our

data: Brownian motion, Ornstein- Uhlenbeck, Early Burst, Trend, Lambda, Kappa, Delta, Drift and White. The AICc (Akaike Information Criterion corrected for small and uneven sample sizes) for each model was reported and Akaike weighting was carried out to rank the models in the order of their fits.

The models all simulate different adaptive evolutionary scenarios, the model that fits the most closely to the observed rate pattern is the best fitted model to the tree. Brownian motion is the most common model, describing a pattern of evolution where the covariance between species values at the tips of the tree, are proportional to the shared history of the taxa (Revell et al., 2008). This is sometimes known as random evolution or a neutral- drift model (Pagel, 1999). Ornstein-Uhlenbeck is a model in which a trait evolves towards an adaptive peak or optimum at some rate and is the simplest of the evolutionary process (Ingram and Mahler, 2013). This model differs from Brownian motion due to the possession of an optimum (Butler and King, 2004). The Early Burst model describes a pattern of evolution in which there is an initial high amount of change and then a slowing of rate later on (Uyeda *et al.,* 2011). Trend is a time dependant model in which shifts occur towards a certain direction. Lambda is a model that tests for phylogenetic signals within the data. This simply means that phylogenetically related organisms are usually similar, so individuals closer together on a tree are usually more similar than ones that are further away (Blomberg et al., 2003). The next model, Kappa, is a punctuated equilibrium model that assesses whether the rate of evolution is gradual or punctuated. Delta describes a model where the rate of evolution is tested to see if it follows a pattern of adaptive radiation or specialisation (Pagel, 1999). The Drift model explains a pattern of evolution where a directional random walk is taken. Finally, white is a model in which there is absolutely no structure in the data.

Three criterions that assess the fit of the model to the tree are outputted from the analysis. Firstly, the Akaike Information Criterion (corrected for small sample sizes) (AICc) is used to

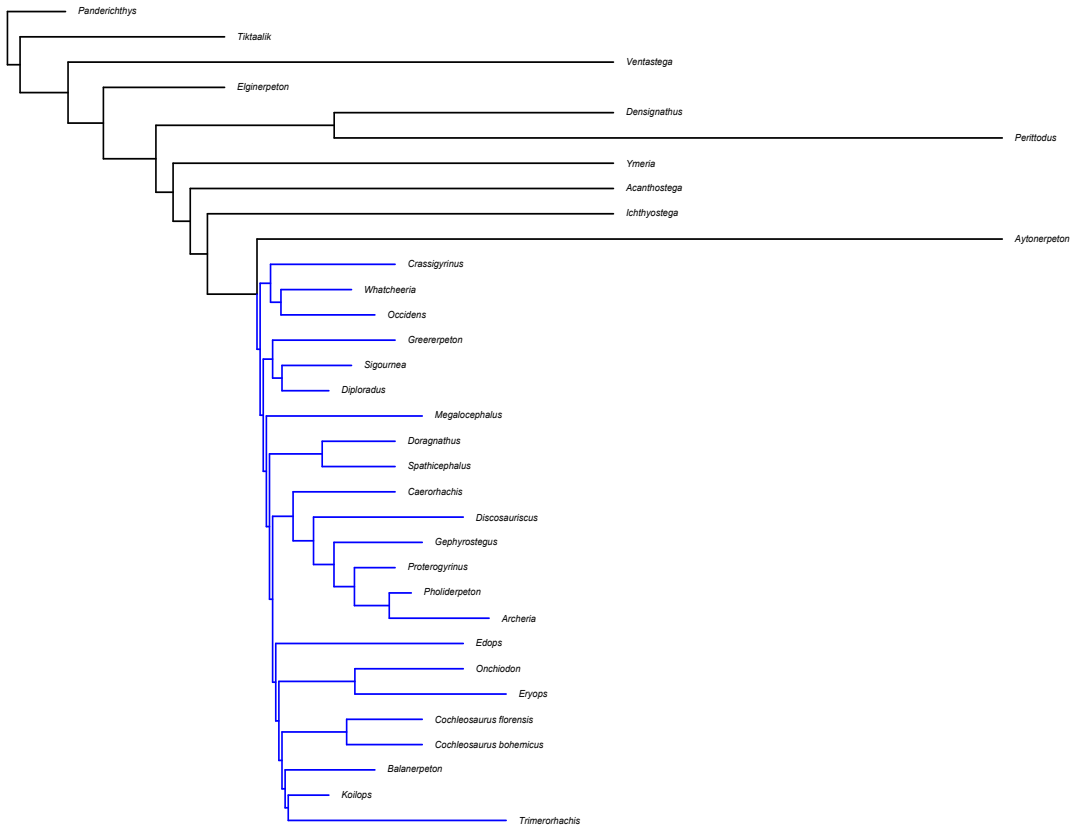
fit models to data with small sample sizes; the lowest AICc shows the best fitting model for the data. The Akaike weight (W) indicates the relative likelihood of the nine models, with the highest weight showing the best model for the data. Finally, Delta shows the difference between ranked models, with a delta value of 0.00 showing the best fitting model to the data.

4.3 Results

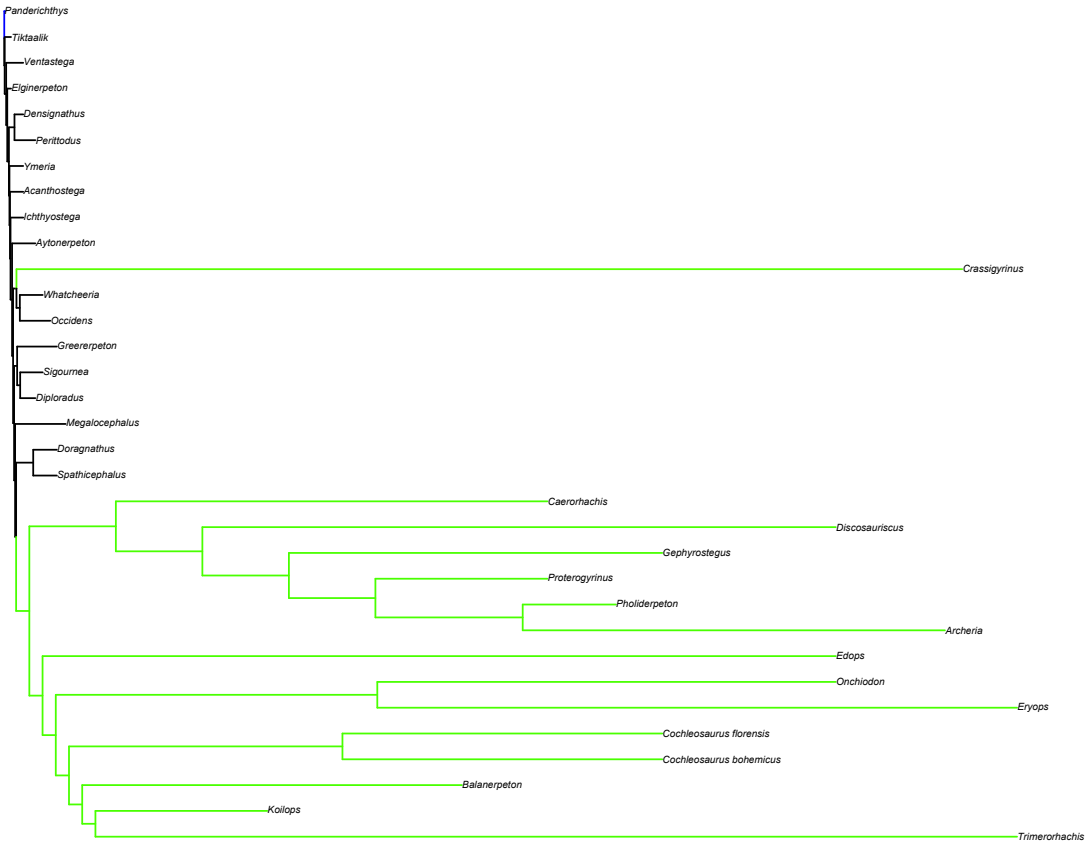
4.3.1 *Motmot*

On the first PCo axis (fig. 14a) there is one instance of a rate shift on the tree with the branches from *Crassigyrinus* to *Trimerorhachis,* showing a reduced rate compared to the background rate (MLRate = 0.096). This shift is found at the end portion of the tree, lining up with the hypothesis of a reduced rate later on in the clades history. The second axis, shows three instances of a rate shift (fig. 14b). Firstly, the branch leading to *Crassigyrinus* has an increased rate (MLRate = 23.4) compared to the rate of the background. The next rate shift is found on the branches from *Caerorhachis* to *Trimerorhachis,* which also shows an increase of rate (MLRate = 13.04). The last instance of rate change is found on the branch from *Panderichthys* to the node leading to *Tiktaalik,* which shows a decrease in rate (MLRate = 1.10^{-8}). This contradicts the hypothesis of an increased rate at the start of the tree. However, these two taxa are tetrapodomorph fishes and display a large range of fish-like characteristics compared to the other taxa, perhaps explaining the reduced rate on these branches. Finally, the third axis displays one instance of rate change, with a high rate (MLRate = 24.64) found on the branch leading to *Greererpeton* (fig. 14c).

a.


Panderichthys
Tiktaalik
Ventastega
Elginerpeton
Densignathus
Perittodus
Ymeria
Acanthostega
Ichthyostega
Aytonerpeton
Crassigyrinus
Whatcheeria
Occidens
Greererpeton
Sigournea
Diploradus
Megalocephalus
Doragnathus
Spathicephalus
Caerorhachis
Discosauriscus
Gephyrostegus
Proterogyrinus
Pholiderpeton
Archeria
Edops
Onchiodon
Eryops
Cochleosaurus florensis
Cochleosaurus bohemicus
Balanerpeton
Koilops
Trimerorhachis

b.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Edops
Onchiodon
Eryops
Cochleosaurus florensis
Cochleosaurus bohemicus
Balanerpeton
Koilops
Trimerorhachis

c.

Fig. 14. Rate analysis in motmot using scores on the first (a), second (b) and third (c) PCo axes. The colours (blue, green and red) indicate that the branch has undergone a change in rate. The length of the coloured branch indicates either an increased rate with a lengthened branch or a decreased rate with a shorter branch relative to the time-calibrated phylogeny.

4.3.2 *Auteur*

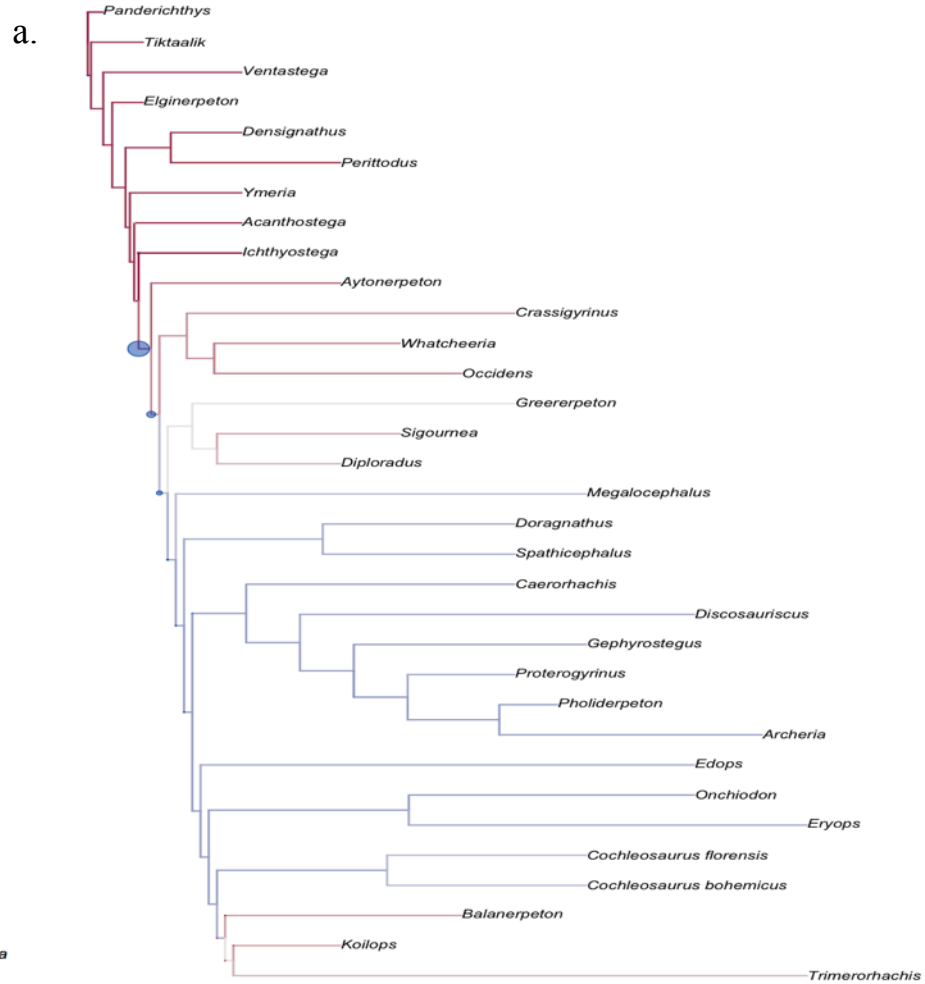
On the first axis 19 instances of rate shifts are found (the largest of these shifts are shown by the large visible circles on the tree in fig. 15a). A high rate compared to the background is retrieved at the top of the tree (fig. 15a). The basal taxa of the group show a high rate compared to the base rate, this corroborates reports of a high rate of evolution at the start of a clade (Colombo *et al.,* 2015). In this case, a high rate at the start of the group indicates that early tetrapods skulls show a burst of morphological rate at the beginning of their group. Rate gradually decreases from the node leading to *Panderichthys* down to the node after the node

leading to *Ichthyostega.* A significant reduction in rate occurs at the node after *Ichthyostega* leading to the less basal stem tetrapods. After this, rate decreases going down the tree until the group of three species at the bottom: *Balanerpeton, Trimerorhachis and Koilops.* The overall decrease of rate from the node leading to *Aytonerpeton* down to *Trimerorhachis* has the largest probability (0.52) and a large down turn of rate (-0.99) on this axis. This again corroborates the hypothesis of an early burst of rate followed by a decrease in rate later on in the clades history.

On the second axis 33 instances of rate shifts are found (fig. 15b.). The largest upturn of rate is found on the node where the splitting event between stem-amphibians and stem-amniotes occurs (probability = 0.40, upturn of rate = 0.99). This increase of rate at one of the most important speciation events during the early tetrapod clade backs up the idea increases of rate being found at the origination of clade. Within the stem-amniotes there is a further upturn in rate at the node that directly leads to the group (probability = 0.29, upturn of rate = 0.99). Due to this the stem-amniotes are found to have a higher rate compared to the base rate than stem-amphibians. Inside the stem-amphibian group there is a small upturn in rate in the node leading to *Balanerpeton, Trimerorhachis and Koilops* as shown on the first axis.

Finally, on the third axis 28 instances of rate shifts are found on the tree (fig. 15c.). The most significant upturn of rate on the third axis is found on the branch leading to *Greererpeton.* This corroborates the increase of rate on the branch leading to *Greerepeton* found in the motmot analysis, showing that this taxon must have underwent a rapid rate of morphological change in the skull. The group containing *Greererpeton, Sigournea and Diploradus* also shows an upturn of rate (probability = 0.20, upturn of rate = 0.98) with the latter two showing a further upturn of rate (probability = 0.15, upturn of rate = 0.91). The first three PCoA coordinates are not congruent for the most part. However, a high rate for the grouping of *Balanerpeton, Trimerohachis* and *Koilops* is shown across the first two axes, showing that there are areas of agreement between the different analyses.

a.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Spathicephalus
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Discosauriscus
Gephyrostegus
Proterogyrinus
Pholiderpeton
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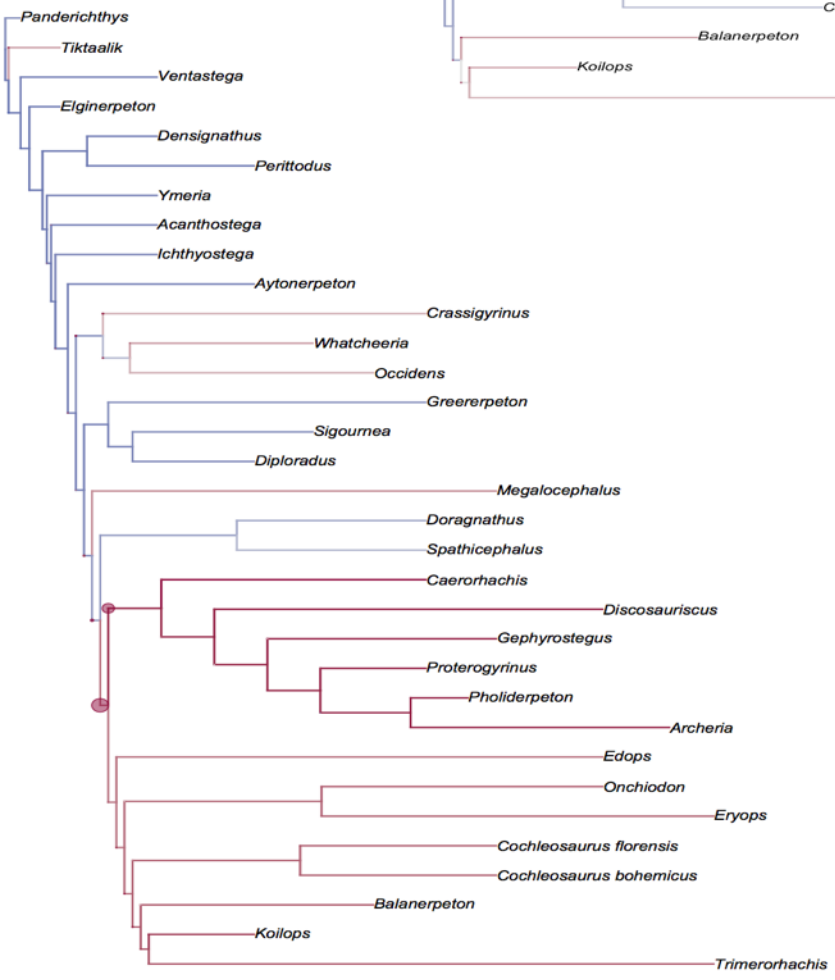
Cochleosaurus bohemicus
Balanerpeton
Koilops
Trimerorhachis

b.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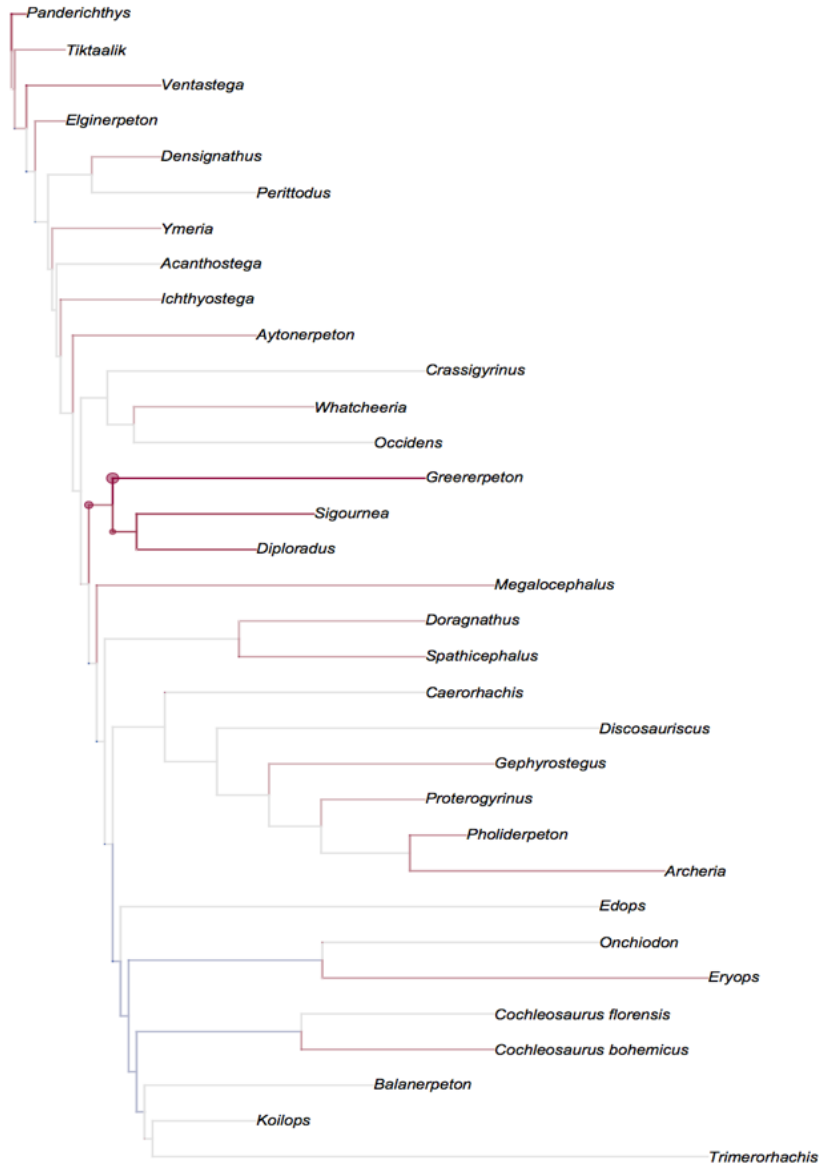

Fig. 15. Auteur plots showing rate of trait change above or below the background rate on the first (a), second (b) and third (c) PCo axes. Grey branches indicate the branch has a rate equal to the background rate of evolution. Branches in red have a higher rate, the deeper the red the higher the rate compared to the background. Branches in blue have a slower rate, the deeper the blue the lower the rate. The size of the circles on nodes represent the probability of the shift to have occurred, the larger the circle the higher the probability of a shift to have occurred. The colour of the circle indicates that at that node there was a change of rate from either high to low or low to high. A darker red the circle indicates there is a higher upturn in rate, whereas a darker blue the circle indicates a downturn in rate.

4.3.3 *Models of Evolution*

PCoA 1

Model	Fit (AICc)	Delta	W
Brownian motion	174.57	24.64	2.6×10^{-5}
Ornstein-Uhlenbeck	187.73	37.80	3.6×10^{-9}
Early burst	**149.93**	**0.00**	**5.8×10^{-1}**
Trend	159.65	9.72	4.5×10^{-3}
Lambda	176.99	27.06	7.7×10^{-7}
Kappa	150.62	0.79	4.1×10^{-1}
Delta	162.87	12.94	9.03×10^{-4}
Drift	167.03	17.10	1.1×10^{-4}
White	185.30	35.37	1.21×10^{-8}

PCoA 2

Model	Fit (AICc)	Delta	W
Brownian motion	141. 31	1.26	1.3 x 10-1
Ornstein-uhlenbeck	143.73	3.69	3.8 x 10-2
Early burst	143.73	3.69	3.8 x 10-2
Trend	**140.05**	**0.00**	**2.4 x 10-1**
Lambda	143.73	3.69	3.8 x 10-2
Kappa	140.07	0.03	2.4 x 10-1
Delta	140.11	0.06	2.3 x 10-1
Drift	143.70	3.65	3.9 x 10-2
White	177.53	37.48	1.8 x 10-9

PCoA 3

Model	Fit (AICc)	Delta	W
Brownian motion	**155.23**	**0.00**	**0.25**
Ornstein-uhlenbeck	157.66	2.43	0.07
Early burst	156.98	1.75	0.10
Trend	156.17	0.94	0.16
Lambda	157.66	2.43	0.07
Kappa	157.66	2.43	0.07
Delta	157.39	2.16	0.08
Drift	156.29	1.06	0.15
White	164.14	8.90	0.003

Table 3. Model fitting of 9 evolutionary models for the first three PCo axes with their Fit (AICc), delta values and Akaike weights (W). Models in bold indicate the best fit for that axis.

The best fitting models for each axis are shown in table 3. On the first PCo axis the best fitting model is early burst. This further backs up the hypothesis of a high rate at the start of the early tetrapod radiation. The second axis shows the Trend model as being the best fitting. This model indicates a directional shift occurring, which coincides with the previous analyses (4.3.1 & 4.3.2) showing that axis two displays a rate shift at the amphibian-amniote split. Finally, the third axis has Brownian Motion as the best fitting model for the data, predicting a random walk pattern of rate for this axis.

4.4 Discussion

The early tetrapod group is an interesting clade in which to study macroevolutionary patterns such as the rate of character state transformations. This is due to the group covering a major evolutionary transition and undergoing numerous morphological changes. Our study uses principal coordinate analysis in order to unravel rate of morphological transformations. These shape transformations with PCo scores cannot be interpreted in the same way as proper morphometric scores. The PCo seeks to capture the position of taxa in a trait space relative to others based on their distances. These PCo scores can be seen as (but are not equivalent to) shape variables. With this we can gain a picture as to the rate at which morphological transformations occurred across the tree.

Ruta *et al.* (2006) found that tetrapods display a decreasing rate of anatomical change over time. Our findings corroborate this in both of the first axes of the Auteur and motmot analyses. However, there are instances in both analyses that contradict this. For example, an increase in rate for the grouping of *Balanerpeton, Koilops* and *Trimerohachis* in two axes of the Auteur analysis. Also, the species *Panderichthys* right at the top of the tree shows a slower rate of evolution on the motmot analysis. This is not surprising, as *Panderichthys* is a tetrapodomorph fish that is close to the tree root (Ahlberg & Clack, 1998). Even due to these anomalies the

pattern of evolutionary rate on the first axis shows that overall the early tetrapod radiation does show a general trend of decreased evolutionary rate over time. The first axis, has Early Burst as the best fitting model; a model in which rate is shown to be high at the start of a clade and decreases during its history (Uyeda *et al.,* 2011). This further backs up the hypothesis of decreasing evolutionary rate in early tetrapods. This also backs up early burst model for an adaptive radiation (Colombo *et al.,* 2015).

The second axis captures a different pattern of evolutionary rate, with species later on in the clades history showing an increased rate compared to the background. This coincides with the amphibian-amniote split on both of the second axes in Auteur and motmot, backing up our hypothesis of a high rate of character state transformations at the split. This high rate can be explained by the colonisation of new ecospace; especially for the stem-amniotes that began to inhabit a fully terrestrial habitat after the split (Ruta *et al.,* 2006). This colonisation of a new habitat would allow an increased amount of exploration of new morphological morphospace and therefore a higher rate of character state transformations (Wagner *et al.,* 2006). Further study, specifically focussed on the split would be beneficial to shed further light upon rate change during this event.

Overall early tetrapod cranial data shows a variety of patterns of the rate of character state transformation over the lineage, however, there are instances of corroboration between analyses. The most important being that both of the periods in which tetrapods invade a new ecospace, at the fish-tetrapod transition and at the amphibian-amniote split, show an increased rate of evolution backing up the idea of character release at the start of a lineage (Wagner *et al.,* 2006). The general rate models, showing the overall evolutionary rate profile, also show this with an early burst model being found indicating a high rate at the start of the lineage and a trend model indicating a directional shift occurring at the amphibian-amniote split. This indicates that early tetrapod cranial morphology undergoes an increase in the rate of character

state transformations at the beginning of the lineages. This is the first time this has been shown in early tetrapod craniums to date.

Our results give an insight into rate of evolution across the early tetrapod clade. However, the results in this study are quite hard to interpret due to the methodology analysing one PCo axis at a time. Due to this the results can give 'contradictory' reports, with one axis showing a different picture of rate than another. A method which can use all of the axes may give a better, more easily interpretable, picture of rate on the tree. However, a method of this nature has not currently been established so the method used here gives us the best picture we can get at the moment. Even due to this, our study has increased our knowledge into the rate of character state transformations using the most comprehensive database of cranial characters to date. A study delving further into this topic, especially with the inclusion of the post cranium, would be the next step towards fully understanding the patterns of rate that the early tetrapod group underwent during the Palaeozoic.

5. General Discussion and Conclusions

Early tetrapods are a diverse group of vertebrates that cover an important evolutionary transition, the transition from water to land (Shubin *et al.,* 2006). Study into the group has been reinvigorated over the past few years due to new early tetrapod discoveries (Ahlberg & Clack 1998; Ahlberg *et al.,* 2000; Smithson & Clack, 2017; Sookias *et al*., 2014). The early tetrapod cranium is a popular complex in regards to character based studies (Ahlberg & Clack 1998; Ruta & Bolt, 2008). However, the group still lacks macroevolutionary studies using the cranium (as well as the rest of the skeleton), such as studies delving into disparity and evolutionary rates. This thesis aimed to fill this gap by creating the largest database of Palaeozoic early tetrapod cranial characters to date in order to retrieve a tree for the group. This tree was time calibrated and then subjected to analyses of disparity and rate.

The phylogenetic analysis, using the parsimony criterion, retrieved a well resolved tree that shows a pattern of taxa similar to what has been found previously (Clack *et al.,* 2016; Ruta & Bolt, 2008; Ruta & Coates, 2007). The tree was time calibrated and showed that due to the inclusion of new taxa the splitting event between the stem-amphibians and stem-amniotes has been pushed backwards. This is earlier than previous estimates, both molecular and character based (Kumar & Hedges, 1998; Reisz & Müller, 2004). The earlier divergence between the stem-amniotes and stem-amphibians is due to the newly discovered Scottish early tetrapods included in this study (Clack *et al.,* 2016). The phylogenetic analysis in this thesis has shed light upon possible new divergence dates in the early tetrapod group as well as a new placement for the four new tetrapods (*Koilops, Diploradus, Perittodus* and *Aytonerpeton).*

The disparity analysis set out to investigate how much morphological similarity or difference is observed by early tetrapod craniums, a type of macroevolutionary study that was surprisingly lacking for the group. The species were found to be significantly clustered in morphospace with

groups (stem-tetrapods, stem-amphibians and stem-amniotes) being significantly separated from each other. The disparity through time plot shows a pattern of taxa with a reduced disparity at the start of the lineage contradicting estimates of an increased amount of disparity at the beginning of a lineage. The analysis of rate shows the opposite to this with an early burst of diversification at the start across all of the analyses (on the first axis). This is in line with previous studies looking at rate in early tetrapods (Ruta *et al.,* 2006). The analysis of rate also shows that at periods in which early tetrapods undergo an invasion of a new ecospace (the fish-tetrapod transition and at the amphibian-amniote split) there is an increased rate of evolution, backing up the idea of character release at the start of a lineage (Wagner *et al.,* 2006). These macroevolutionary studies have shed light upon how early tetrapods evolved in the Palaeozoic, an area in which lacks macroevolutionary study.

The analyses in this thesis give us an insight into the early tetrapod groups relationships and crucially shows that the splitting event, when the tetrapod clade diverges into two separate lineages (stem-amphibians and stem-amniotes), is potentially further back than previously thought. This has implications not only for the early tetrapod group but for the whole of the amniote and amphibian lineages. This study of early tetrapod cranial characters also sheds light upon the disparity and rate of evolution in a group that is seriously lacking in macroevolutionary study. This shows that the early tetrapods do have the potential to have an early burst of diversification at the start of the lineage, as well as another burst during the divergence between the stem-amniotes and stem-amphibians. Together these analyses push the field of early tetrapod study forward immensely by creating the most comprehensive dataset and phylogeny using early tetrapod cranial characters ever assembled via modern modelling methods. Due to this the results from this project, as well as the database compiled can be used as a stepping stone in order to increase understanding into the early tetrapod group as a whole. However, in order to get a more in depth picture of the group the next step would be to include postcranial characters into the database as well as expanding the taxa list to include even more of the groups diversity.

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